

Understanding and managing Hop Latent Viroid in cannabis (*Cannabis sativa* L.)

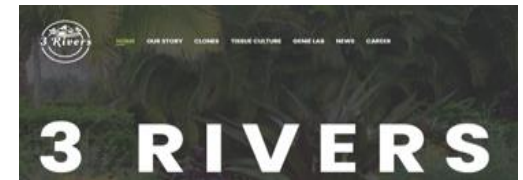
Zamir K. Punja



SIMON FRASER
UNIVERSITY



PURE SUNFARMS



A&L Canada Laboratories Inc.



Agriculture and
Agri-Food Canada

Presentation Outline

--The **seven** “S”s of this disease –

- Symptoms
- Severity
- Significance
- Spread
- Survival
- Stability
- Sanitation

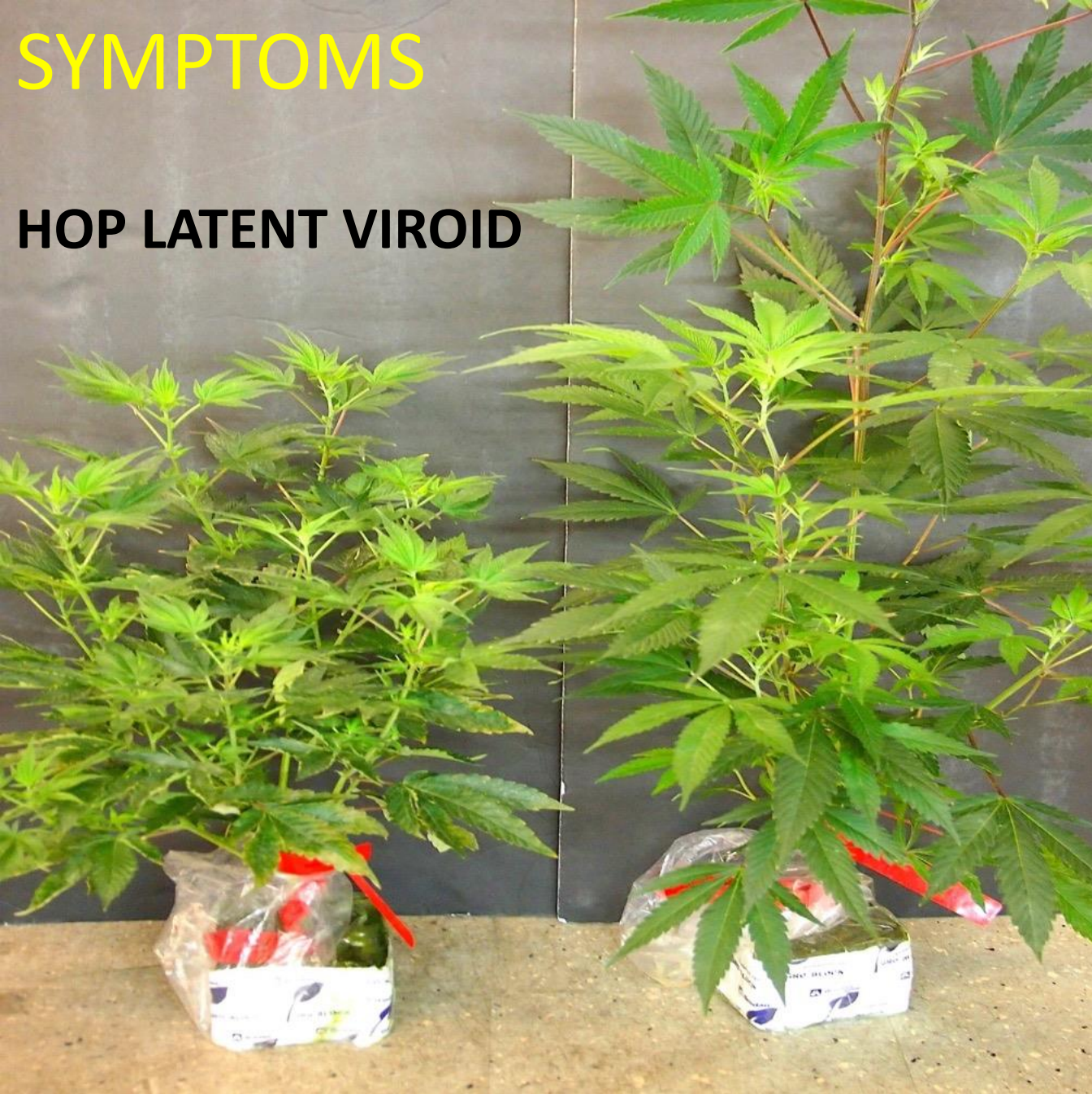
All at @ SFU !

Hop latent viroid has spread into greenhouse production of cannabis in Canada



SYMPTOMS

HOP LATENT VIROID



INFECTED

HEALTHY

Vegetative plants, pre-flower, of a highly susceptible genotype of cannabis, shows obvious stunting

SYMPTOMS - On flowering plants (2 week

Infected (left) Healthy (right)



SYMPTOMS

INFECTED



HEALTHY



SEVERITY

Effect on inflorescence size



SEVERITY

Effect on root growth – reduced root volume and length

Healthy

Infected



Healthy

Infected

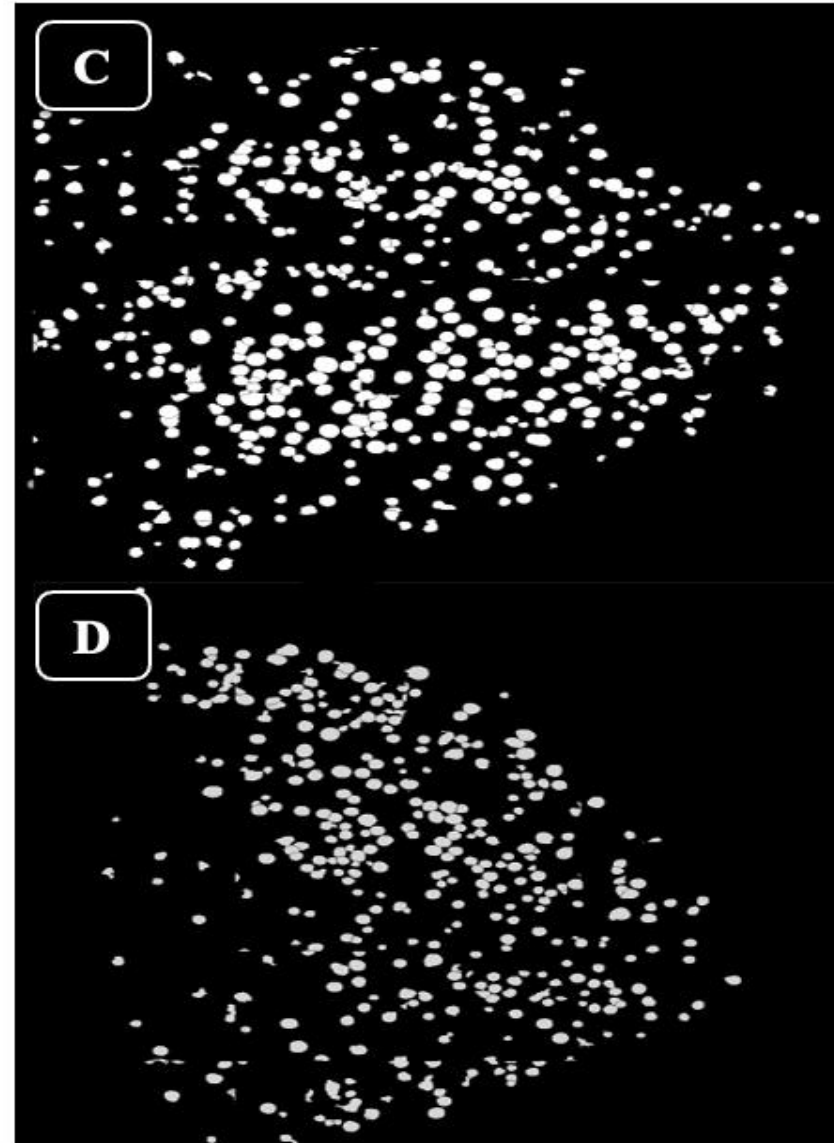
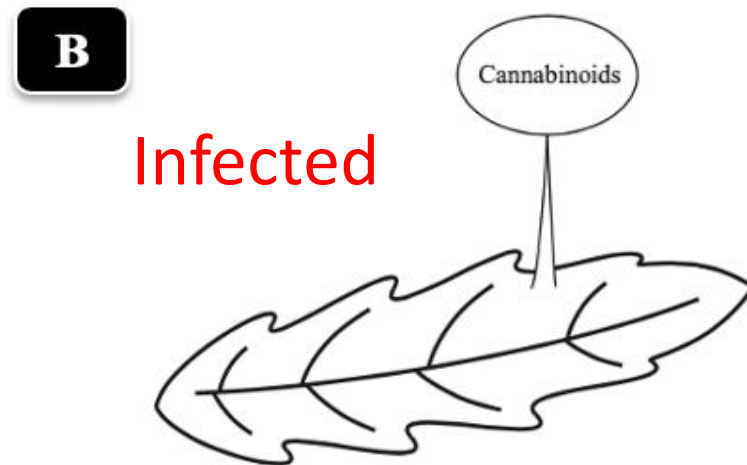
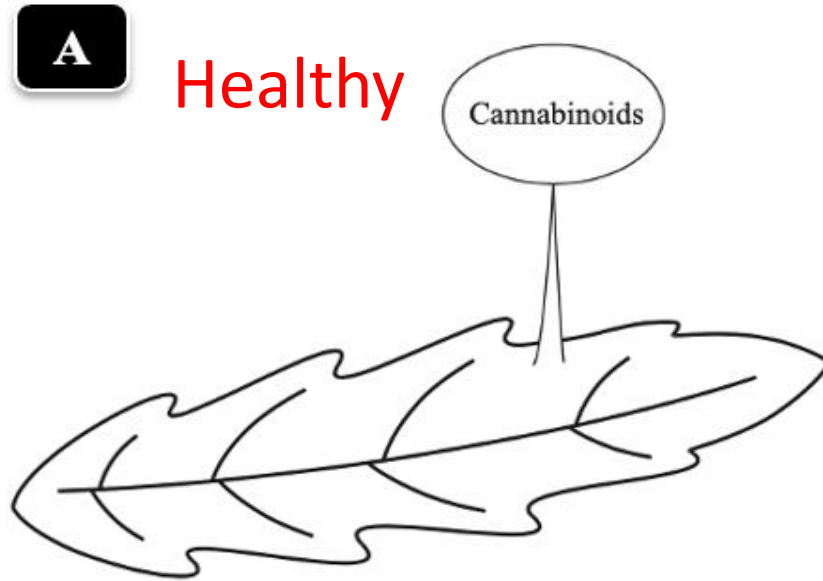


SEVERITY - Hop latent viroid significantly reduces flower size

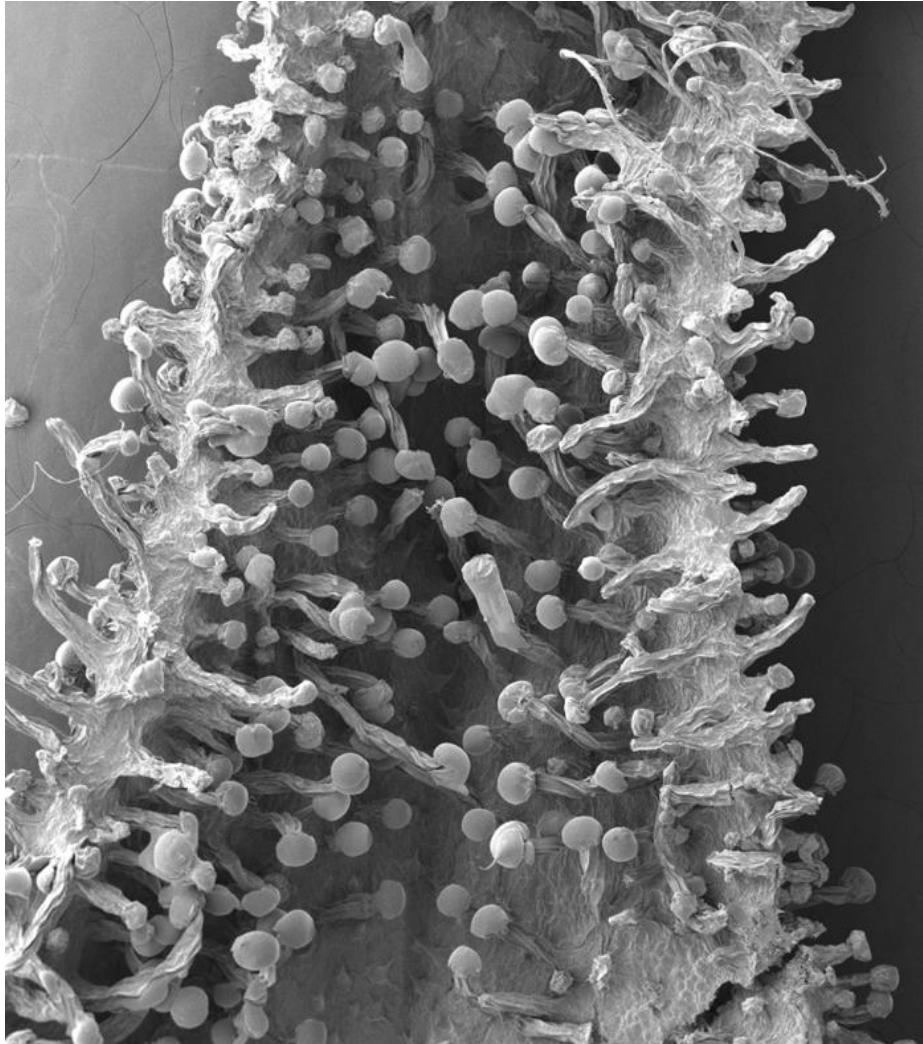


This has economic impact as well as a potential downside to cannabis product quality

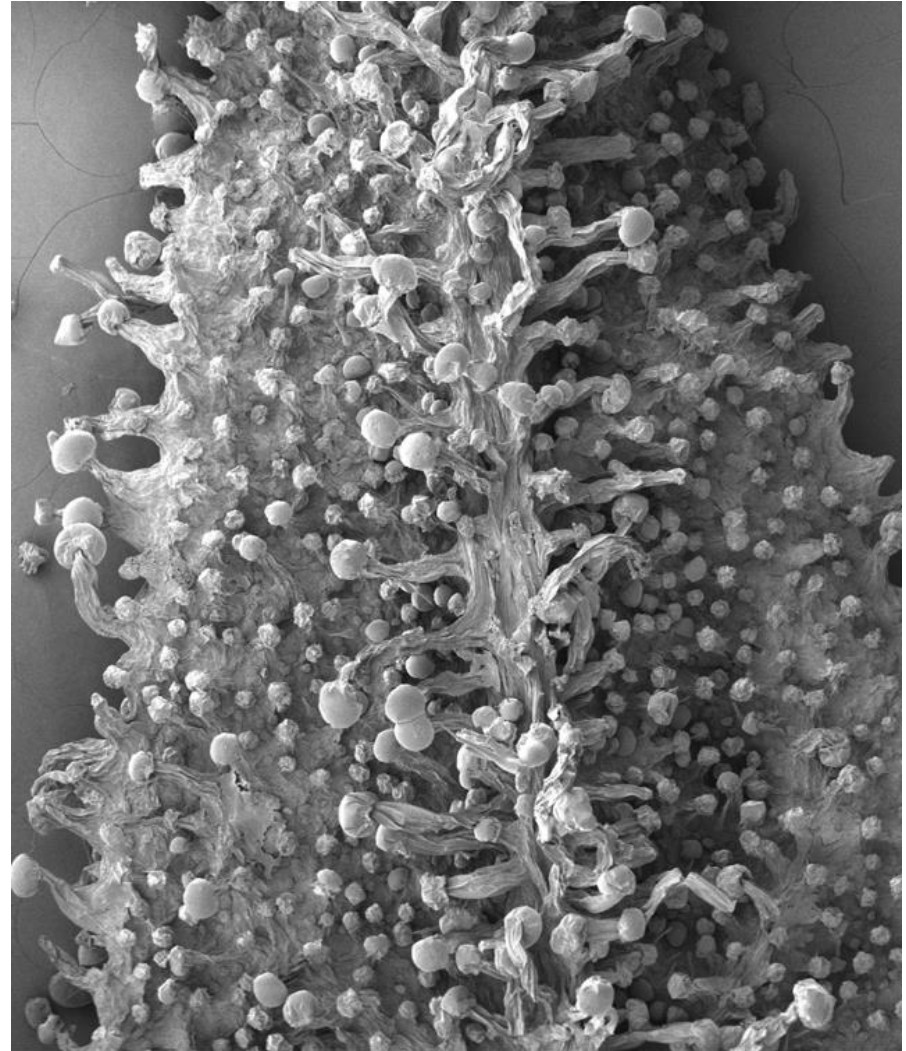
Significance – reduced bract size and trichome size



SIGNIFICANCE – Reduced trichome development on bracts



ASYMPTOMATIC



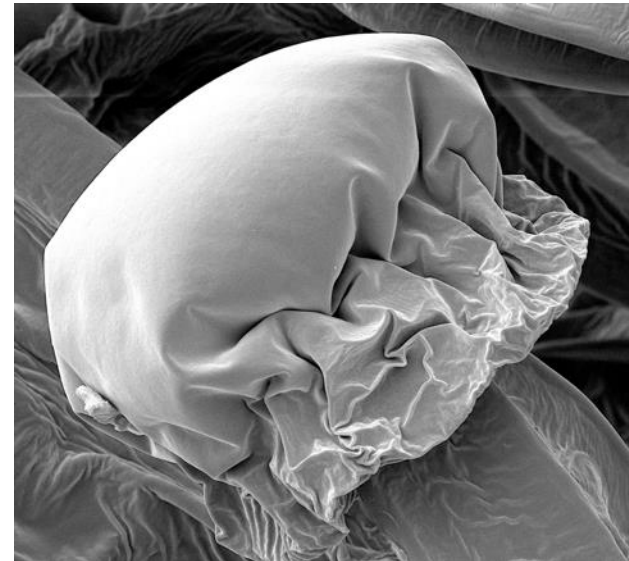
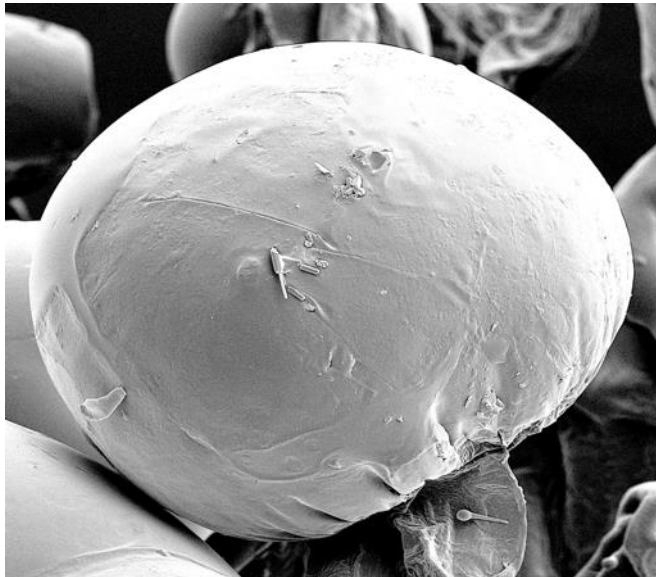
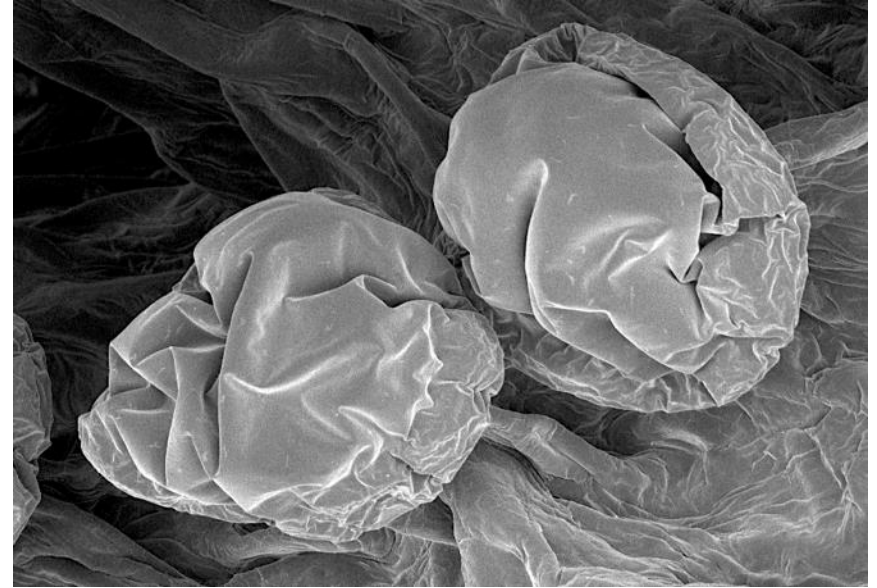
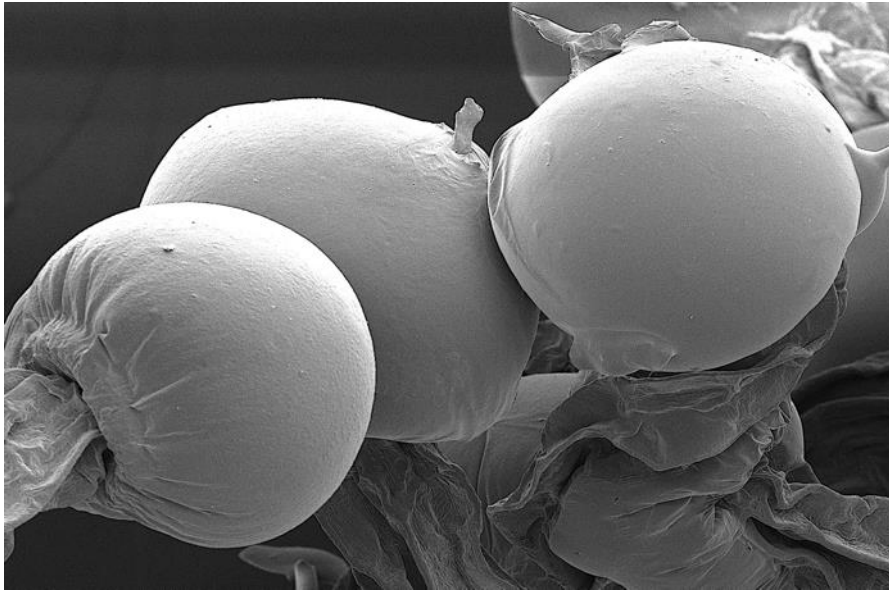
SYMPTOMATIC

SIGNIFICANCE

Effect on trichome gland size/volume

ASYMPTOMATIC

SYMPTOMATIC



SIGNIFICANT REDUCTION IN THC AND TERPENES

HpLVd negative

27 % THC

1.2 %
terpenes

HpLVd
positive

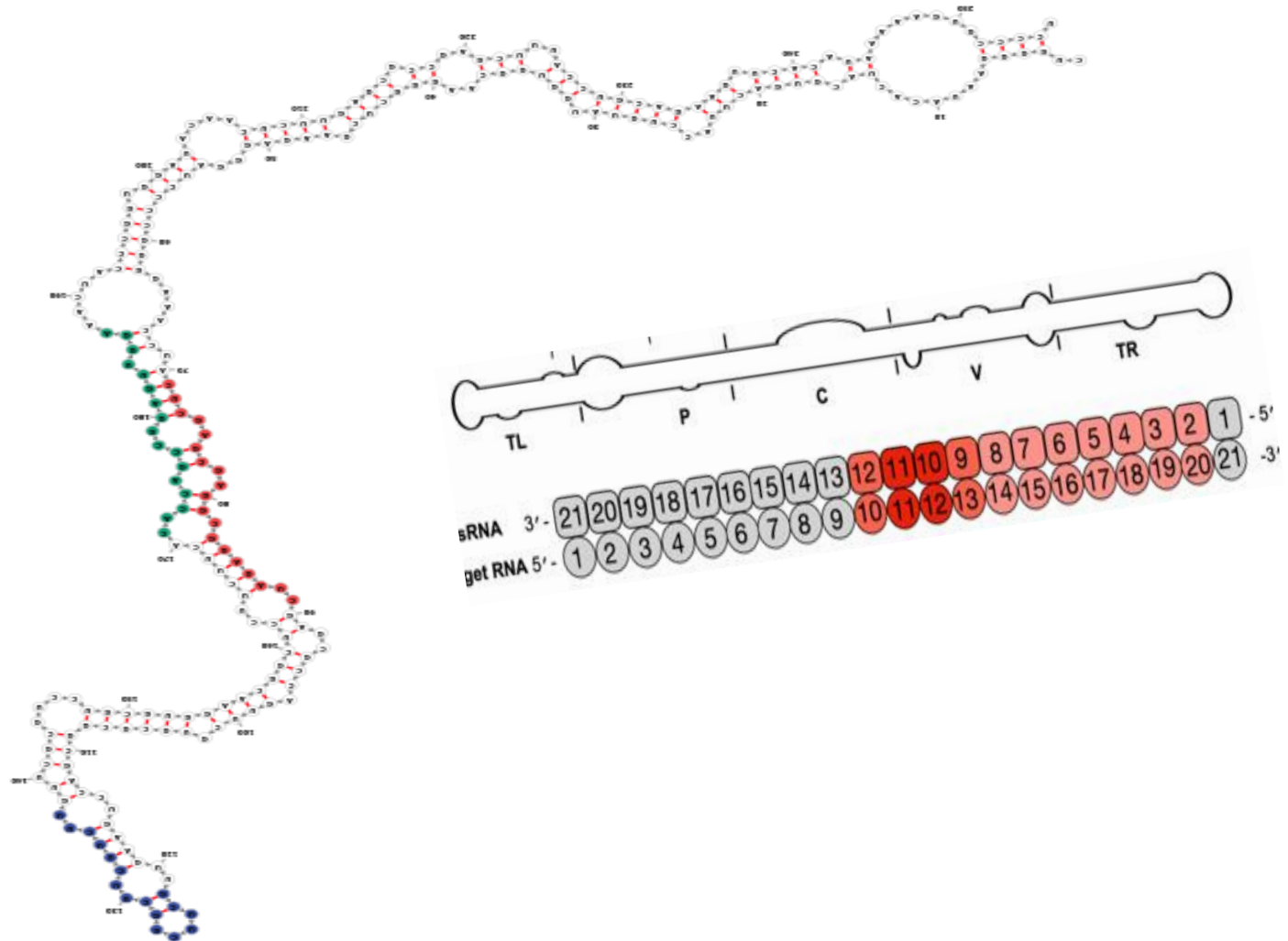
15 % THC

1.1 %
terpenes



SIGNIFICANCE ---- WHAT IS IT ????

Hop Latent Viroid = 256 nt of RNA sequence



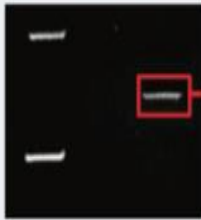
Hop Latent viroid positive detection by RT-PCR with specific primers

Inoculum source

From infected tissues

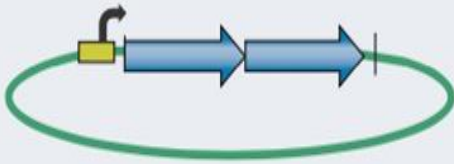


Viroid purification



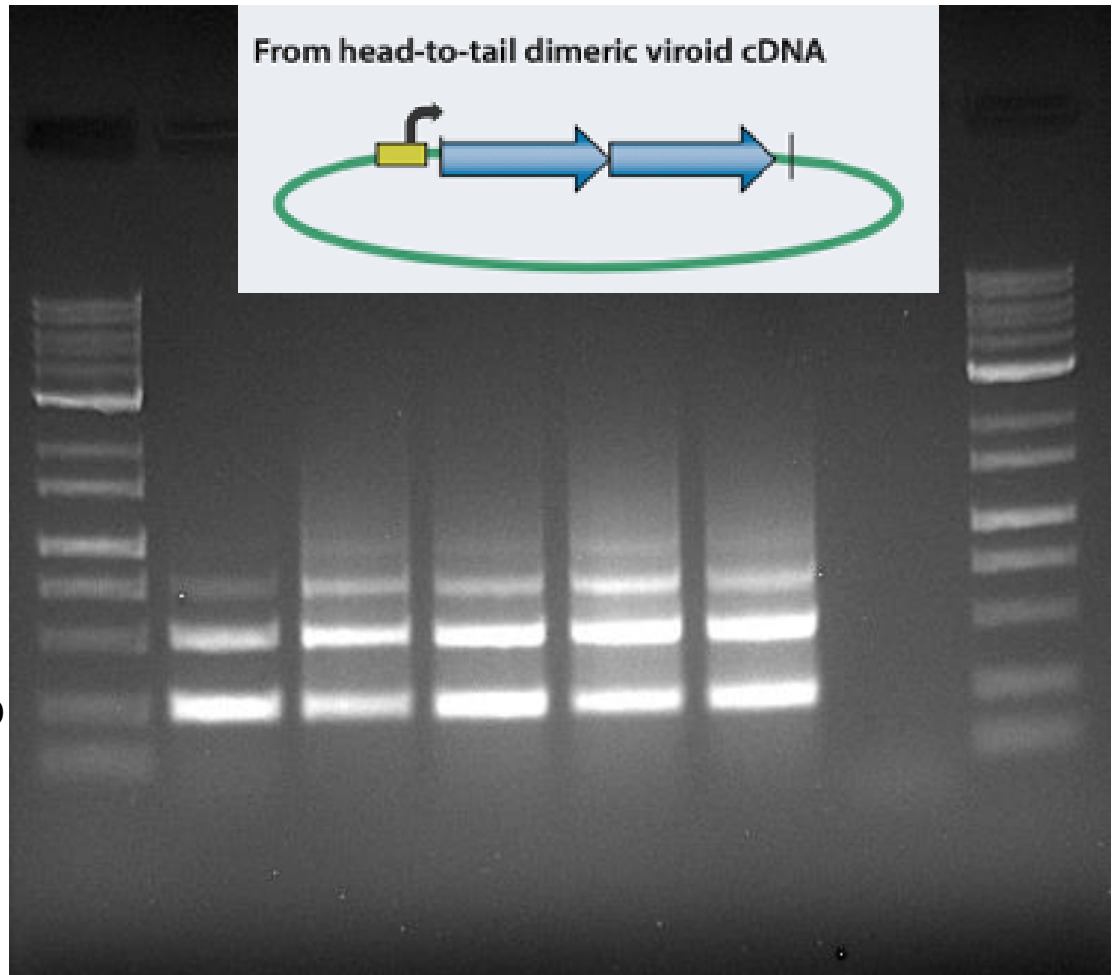
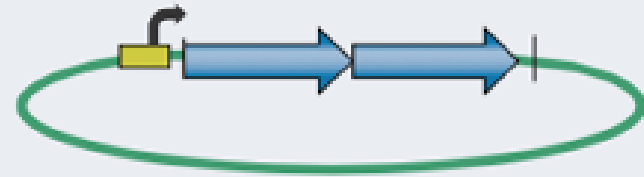
Circular
viroid RNA

From head-to-tail dimeric viroid cDNA



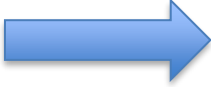
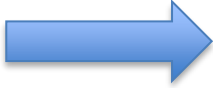
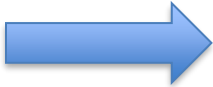
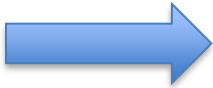
256 bp

From head-to-tail dimeric viroid cDNA

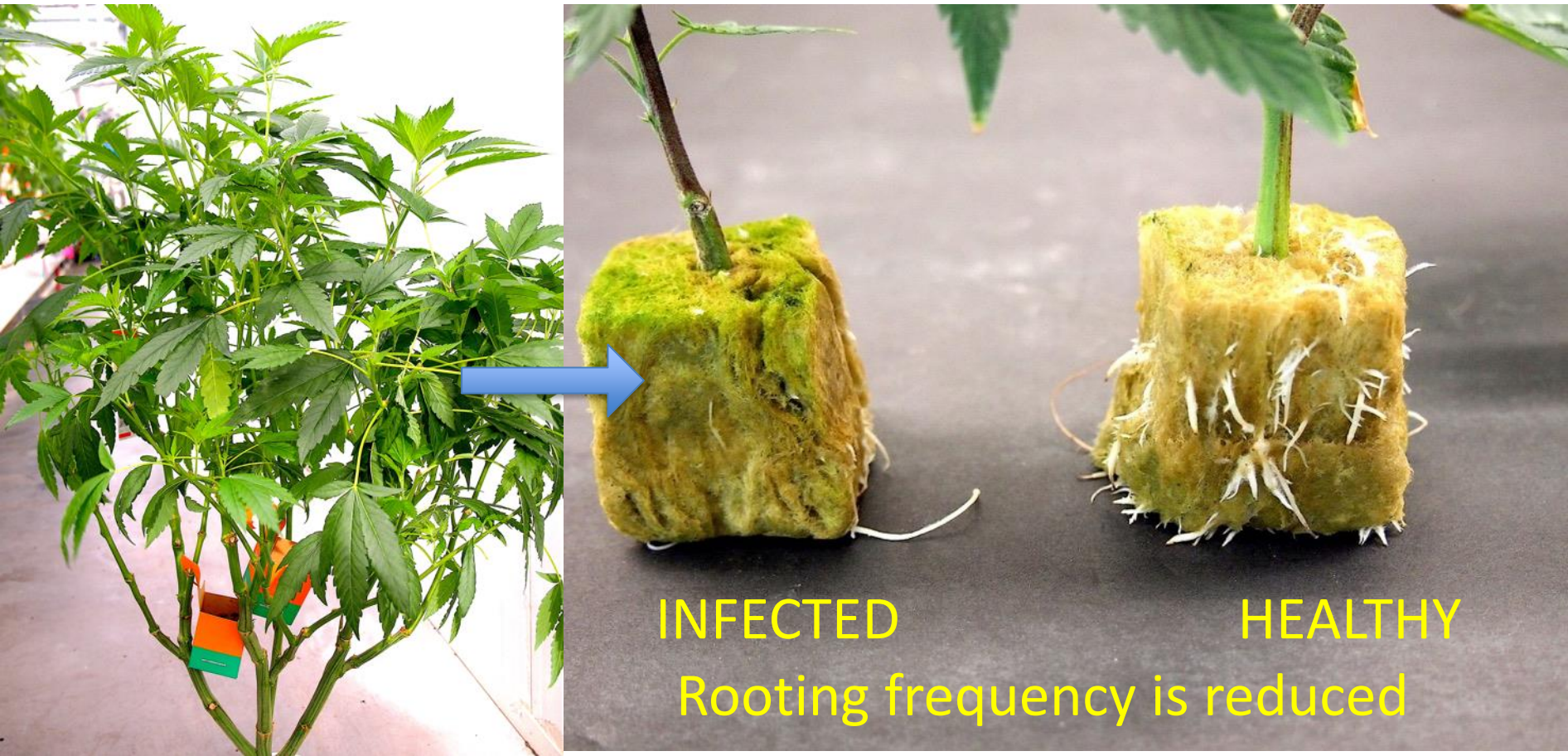


L +ve lower middle top roots -ve L
leaf leaf leaf

SPREAD

- Mother plant  Cuttings
- Pruning wounds  Stem infection
- Stems  Roots
- Roots  Systemic infection
- Spread through water to infect roots
- Spread into flowers (female and male)
- Spread into trichomes
- Spread into seeds

SPREAD - Cuttings taken from Hop Latent viroid infected mother plants are **infected**

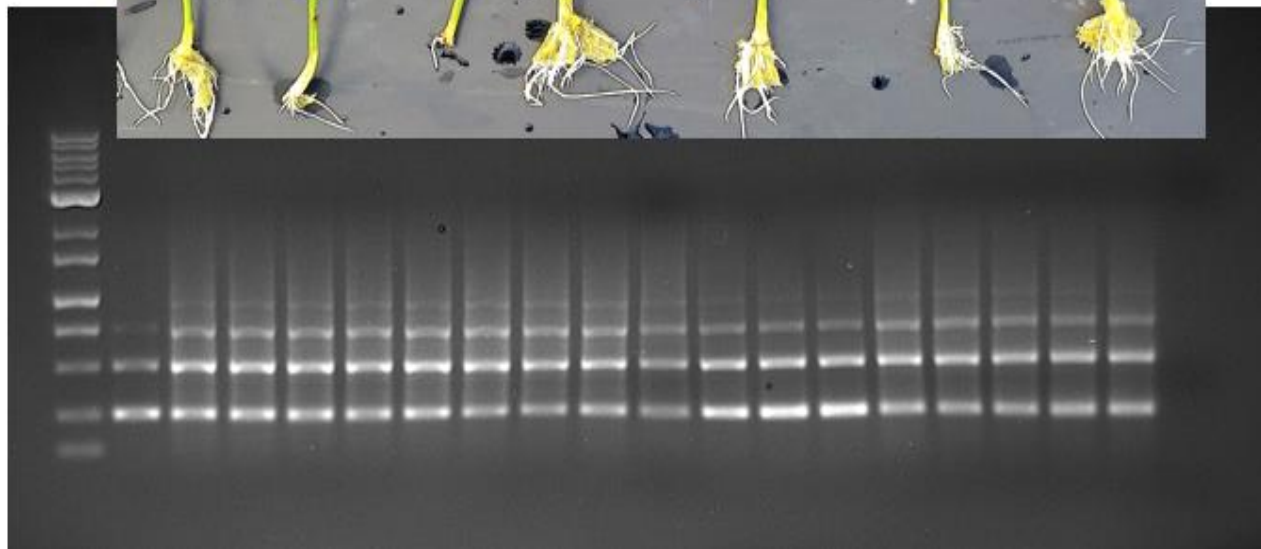


LEFT – Cutting from infected plant. RIGHT – Cutting from healthy plant

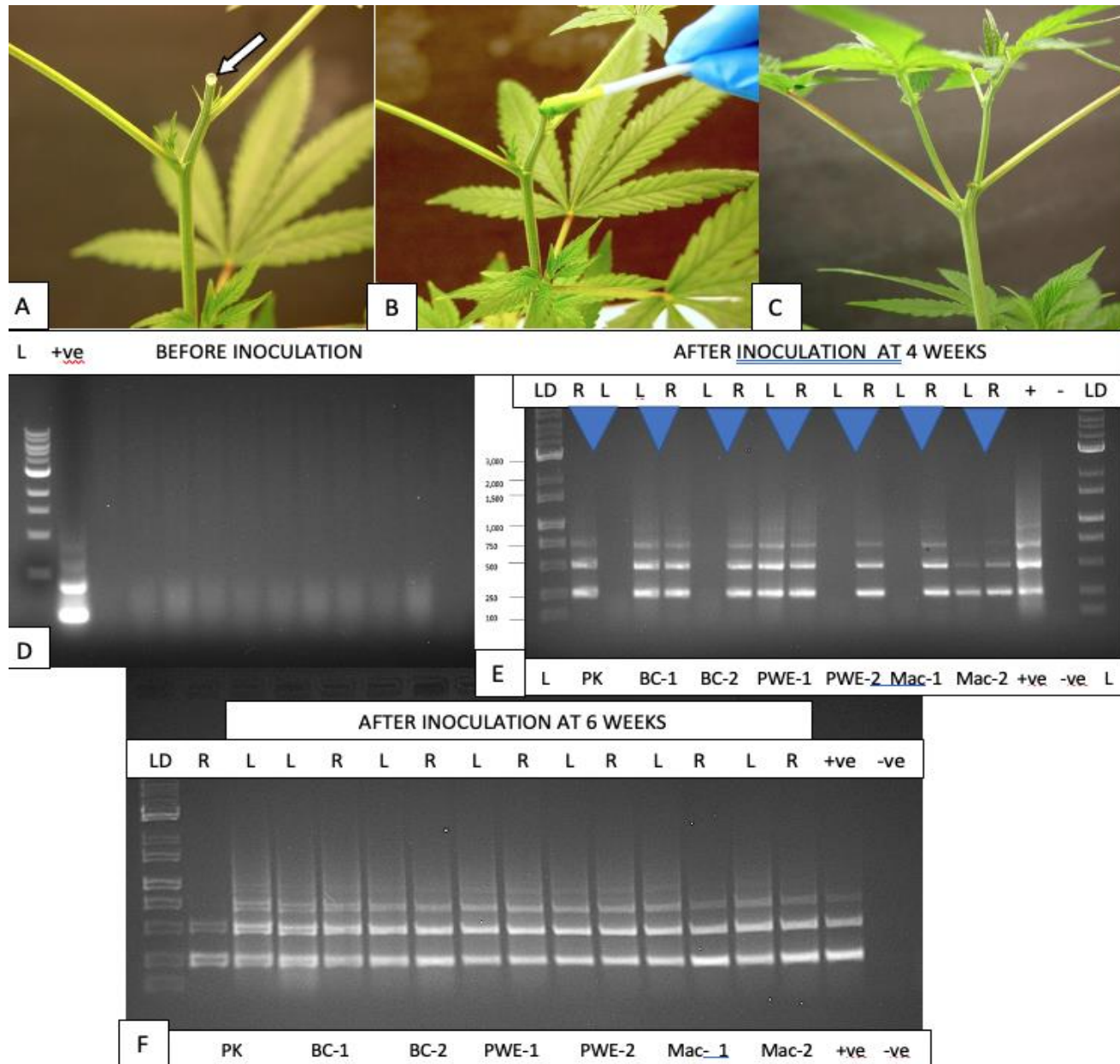
MBD

PWD

PNK



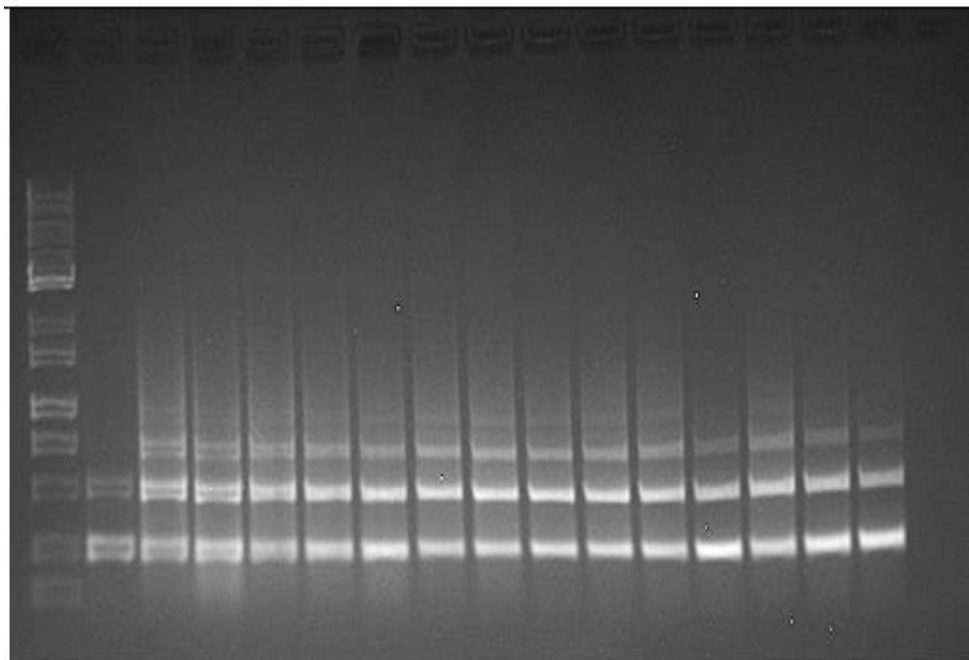
SPREAD----- Artificial transmission of Hop Latent viroid on exposed stem cuts



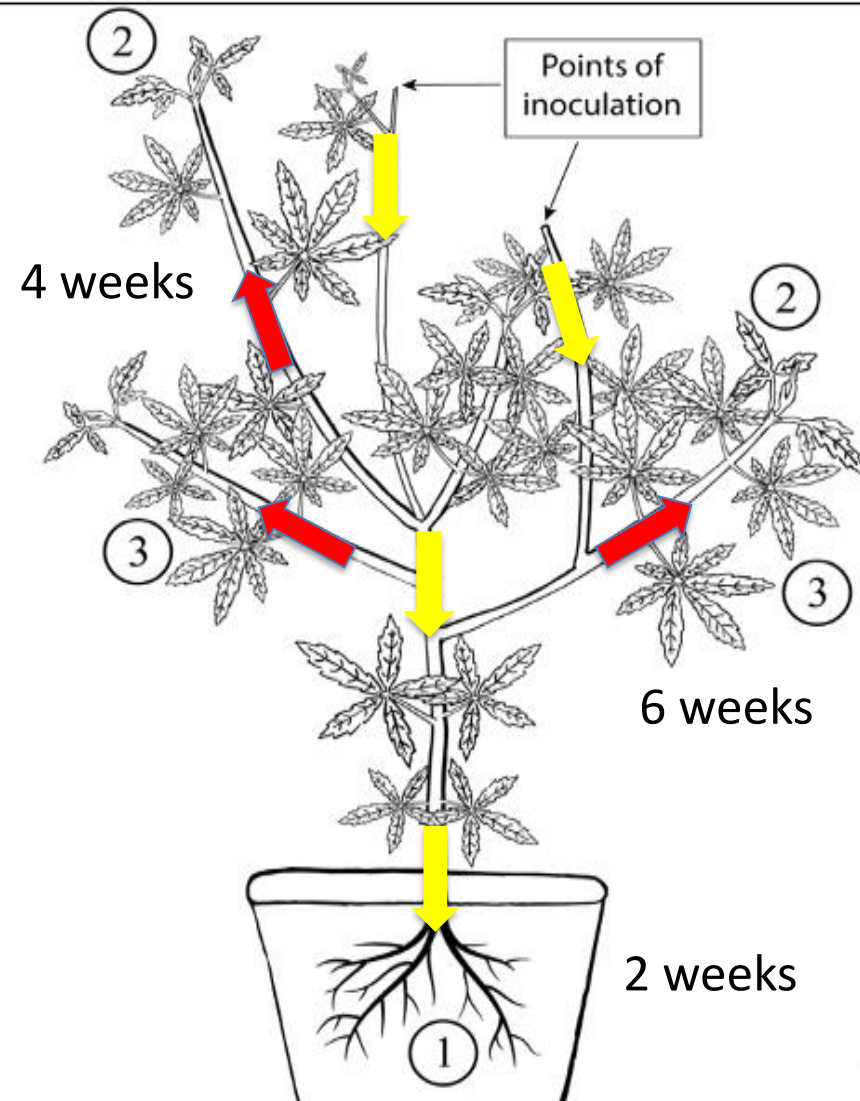
SPREAD ----- Found in the roots in 2 weeks, in the young leaves in 4 weeks, throughout the plant in 5-6 weeks

AFTER INOCULATION AT 6 WEEKS

L PK BC-1 BC-2 PWE-1 PWE-2 Mac- 1 Mac-2 +ve -ve



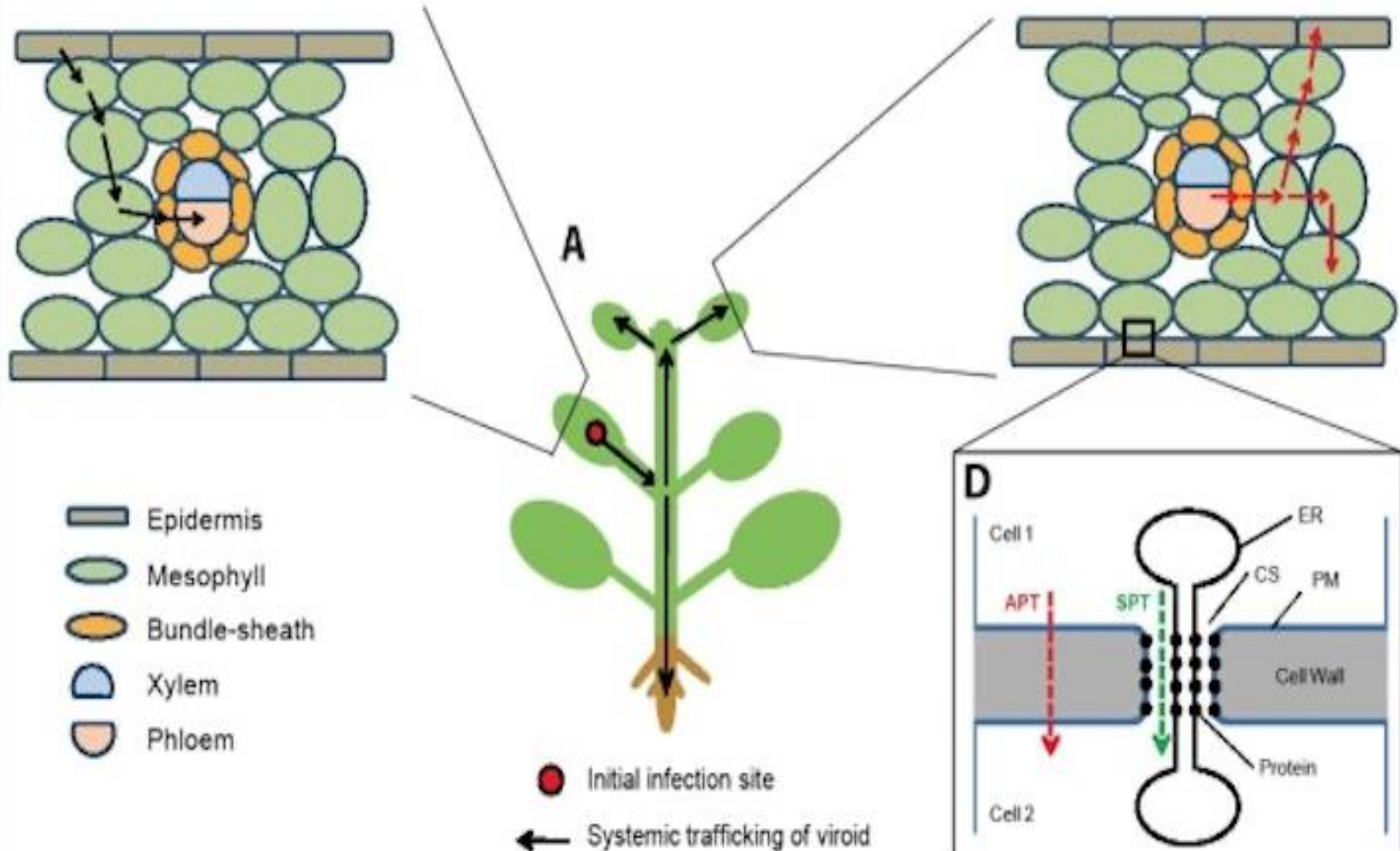
L R L L R L R L R L R L R L R +ve



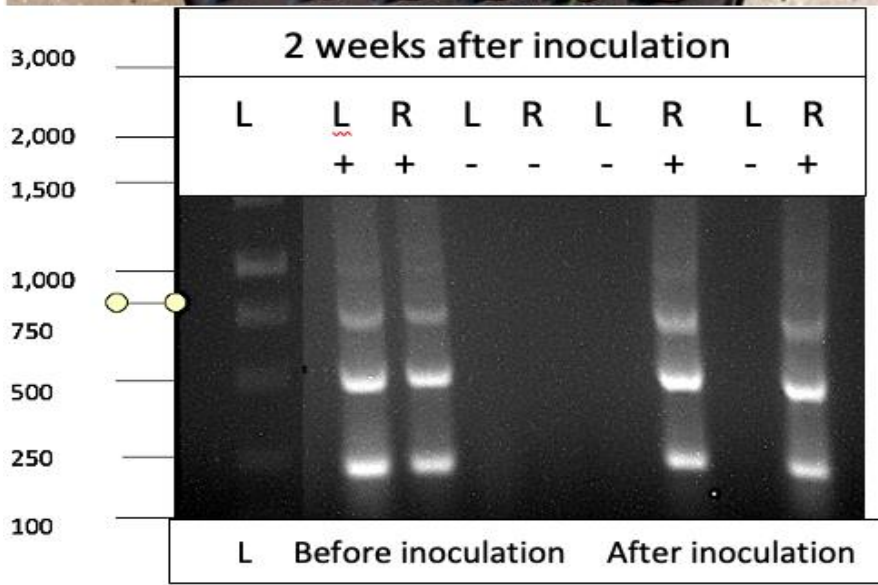
Spread of Hop Latent viroid

B Entry into phloem →

C Exit from phloem →



SPREAD ----- Transmission occurs through hydroponic nutrient solution and water

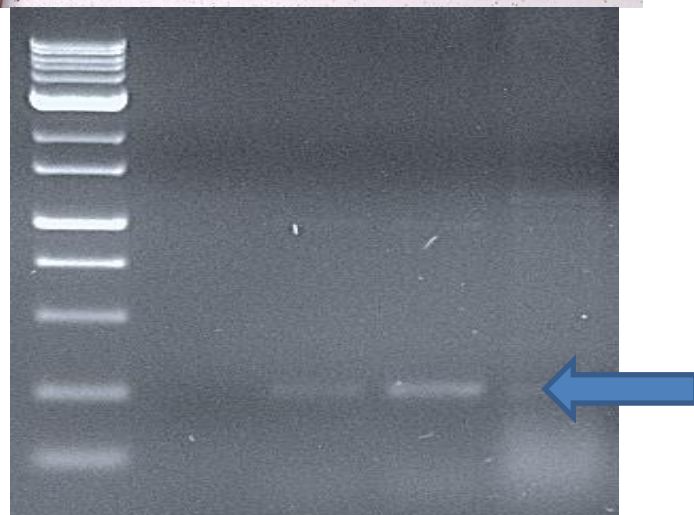
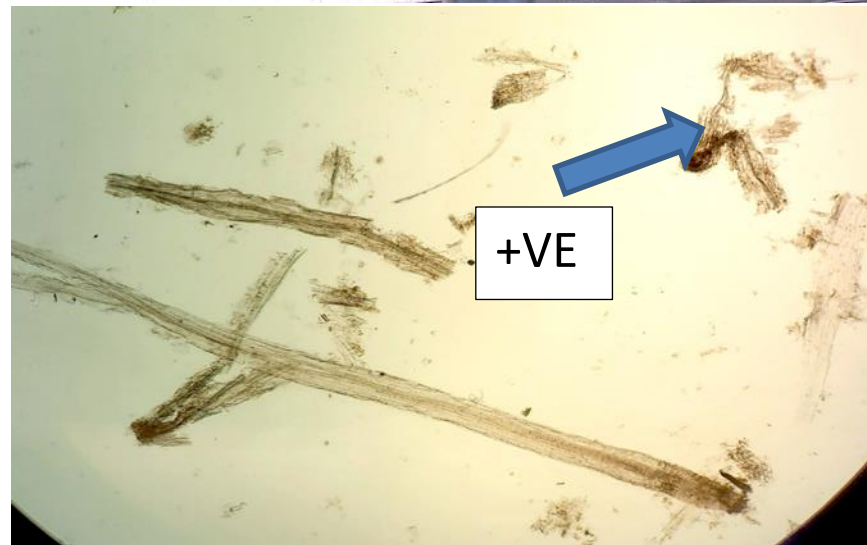
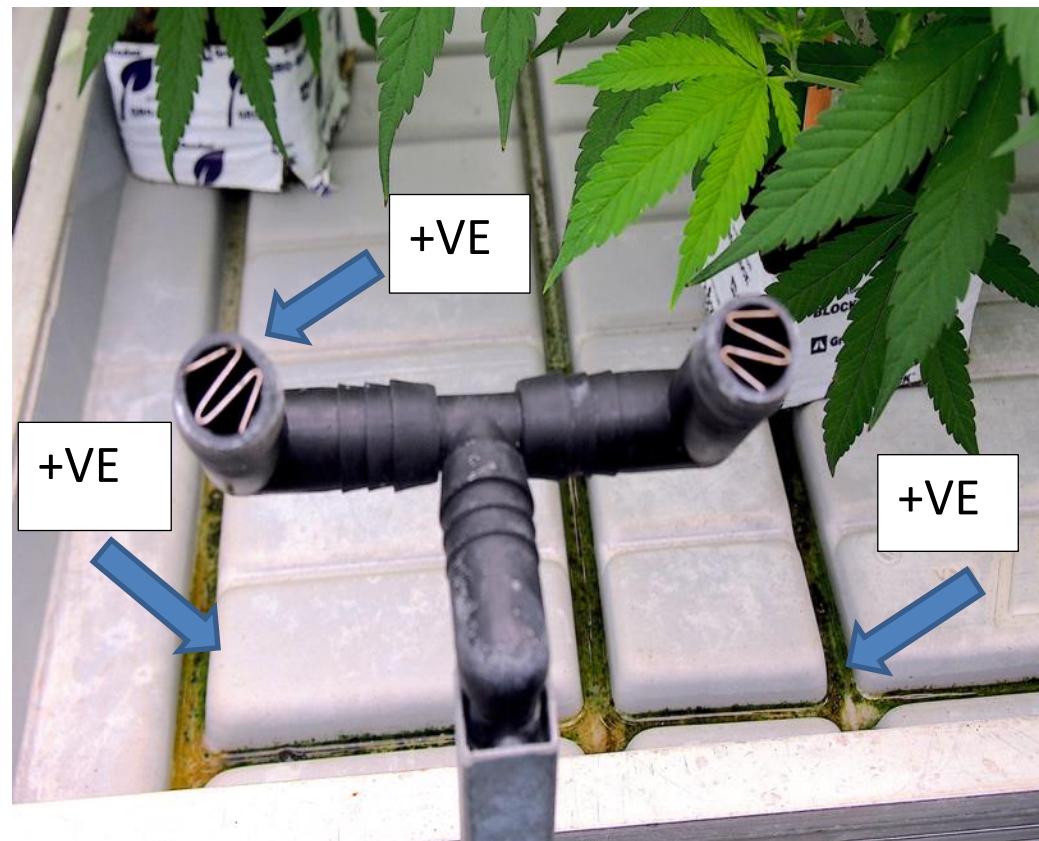
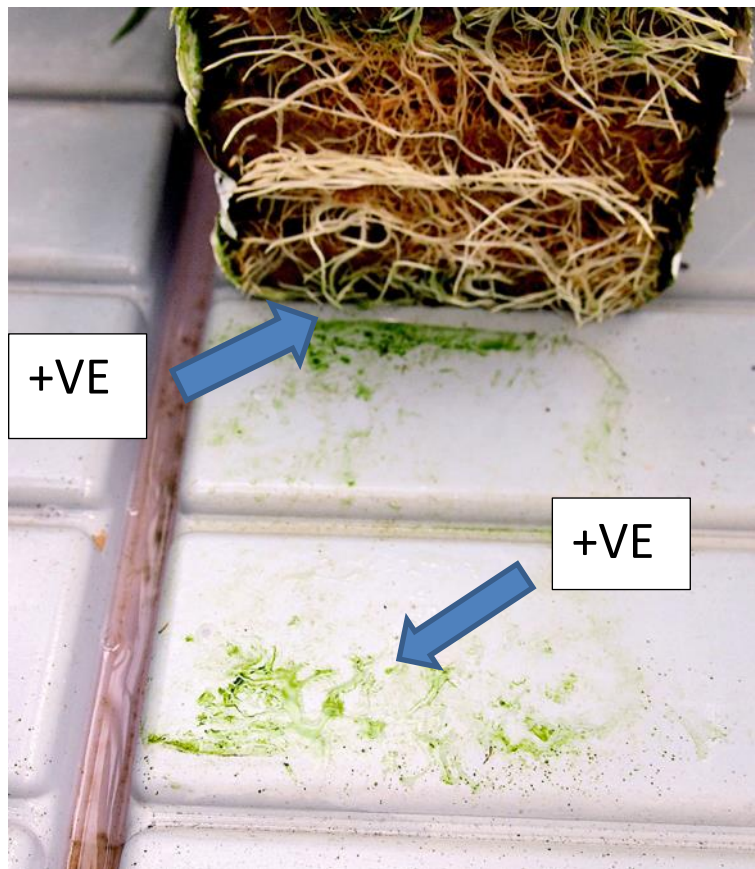


The viroid was detected after 2 weeks of exposure of healthy roots to a solution containing infected roots. There was no physical root contact.

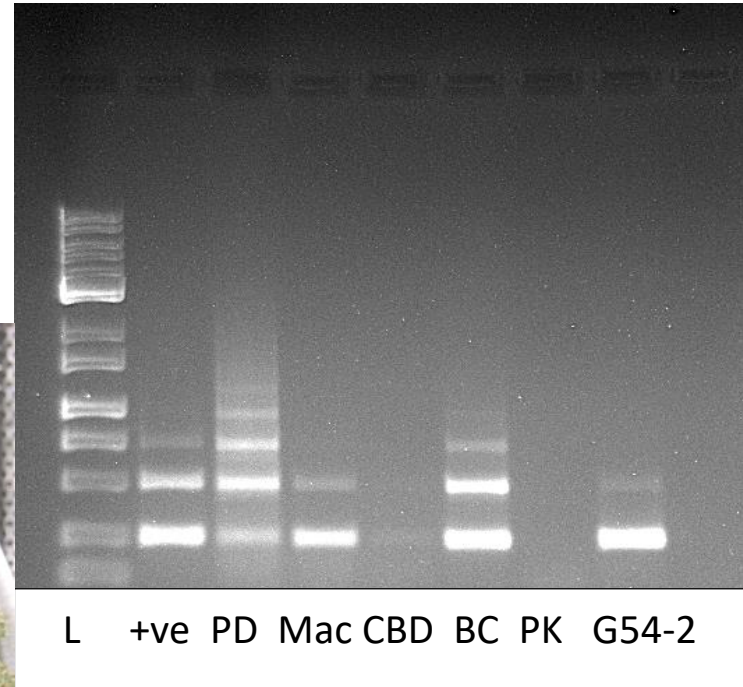
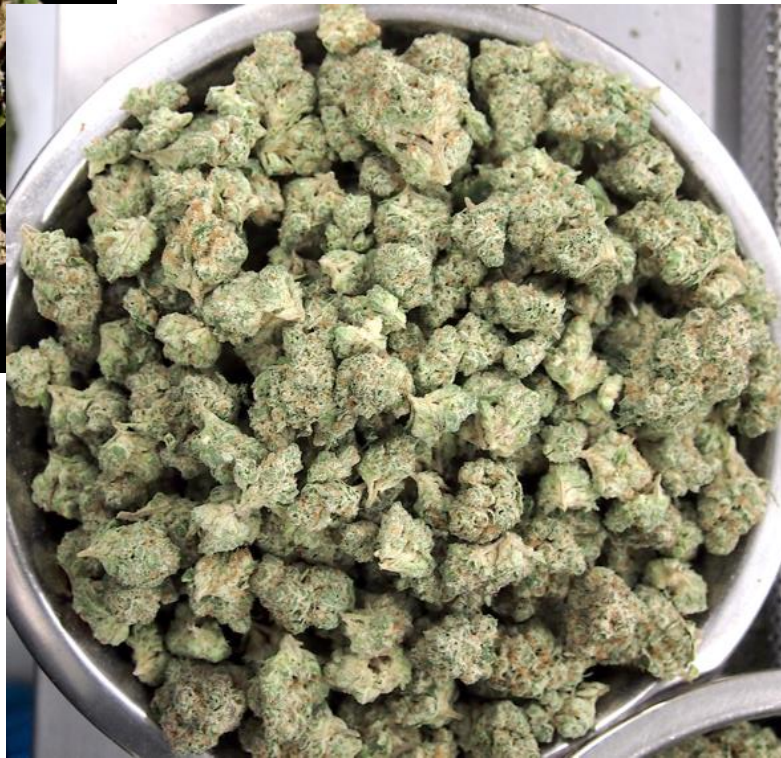
SPREAD ----- Root to root transmission through
recirculating water 20 % transmission
rate



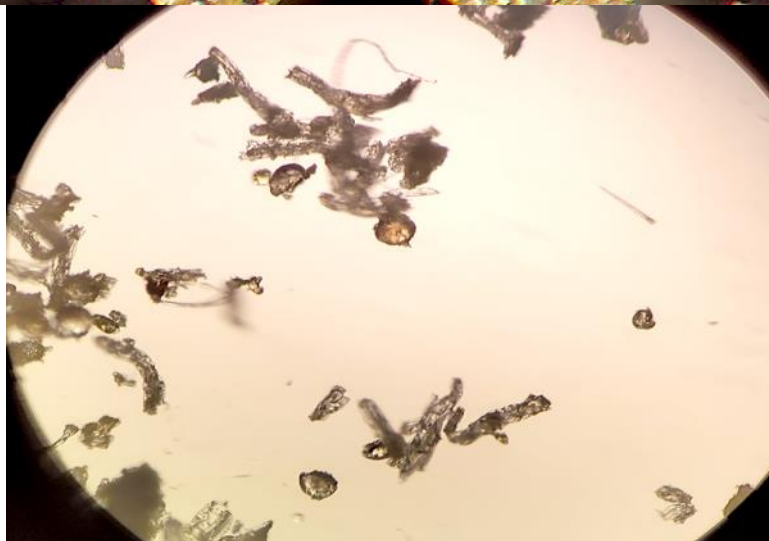
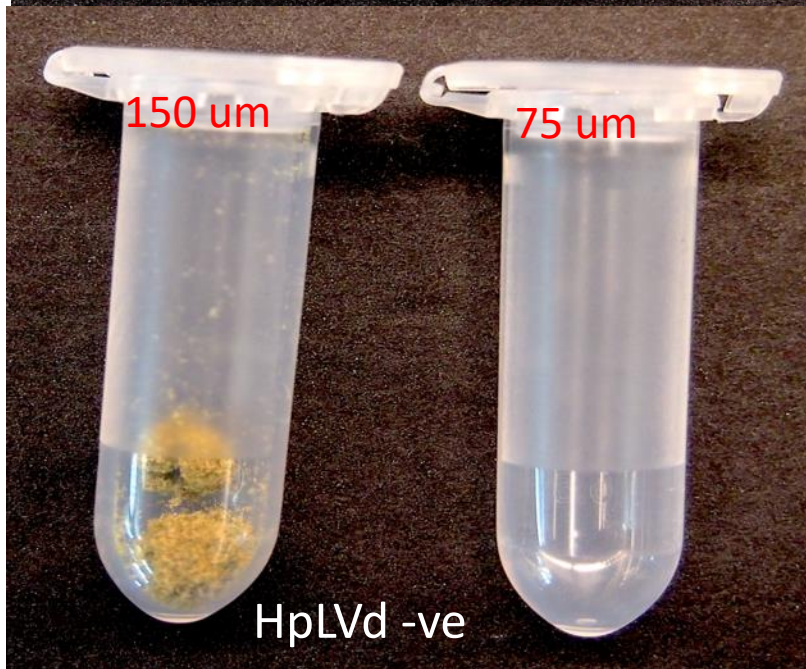
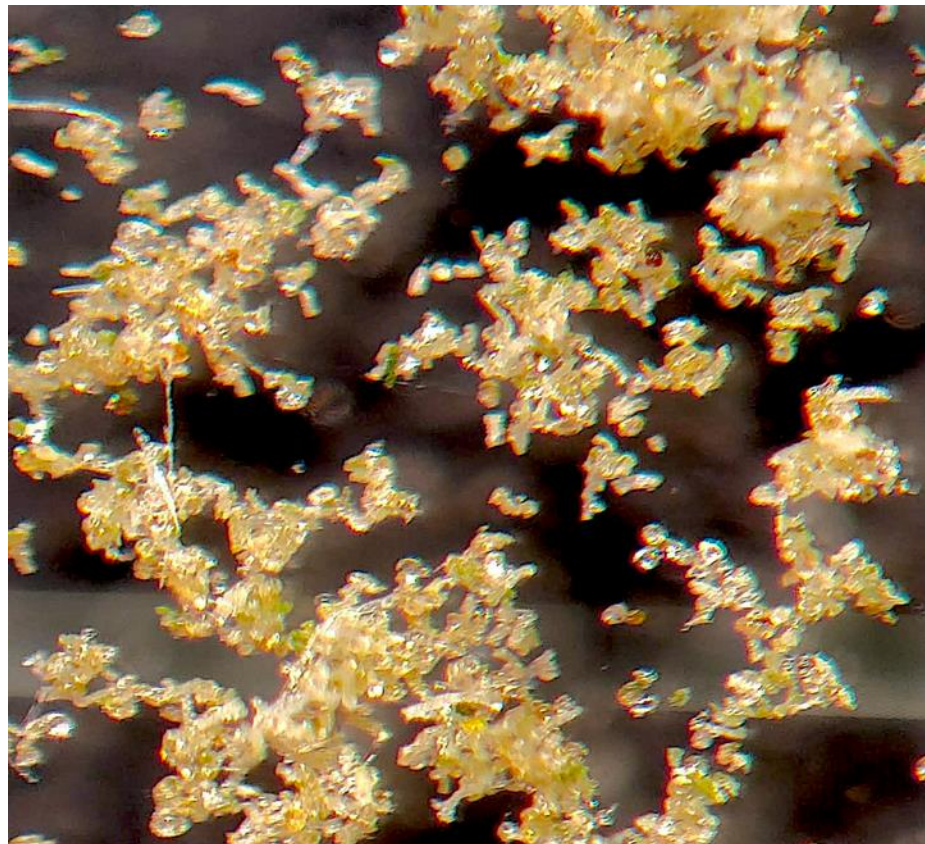
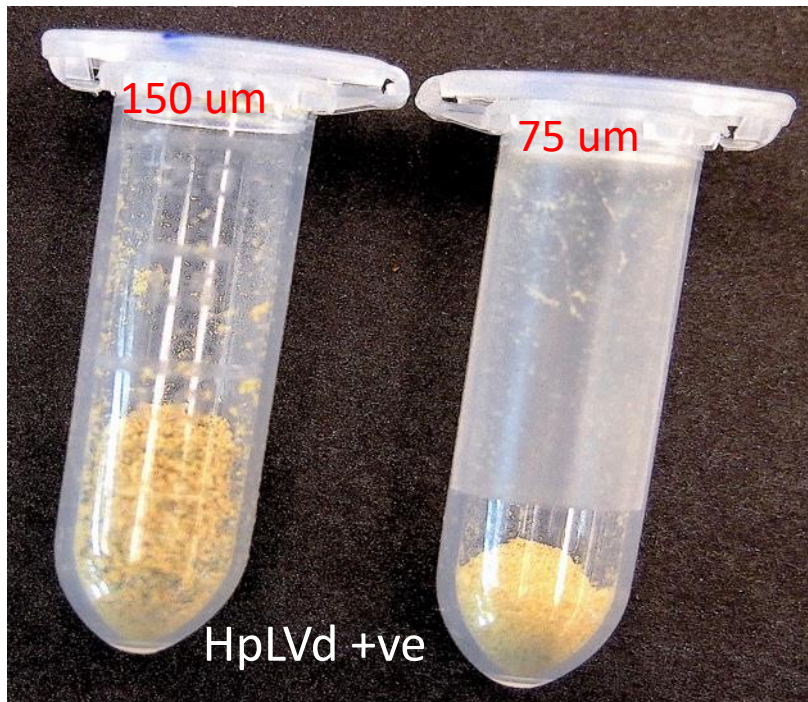
Hop latent viroid was detected in recirculating nutrient
solution



SPREAD ----- Hop Latent viroid is found in dried flower samples

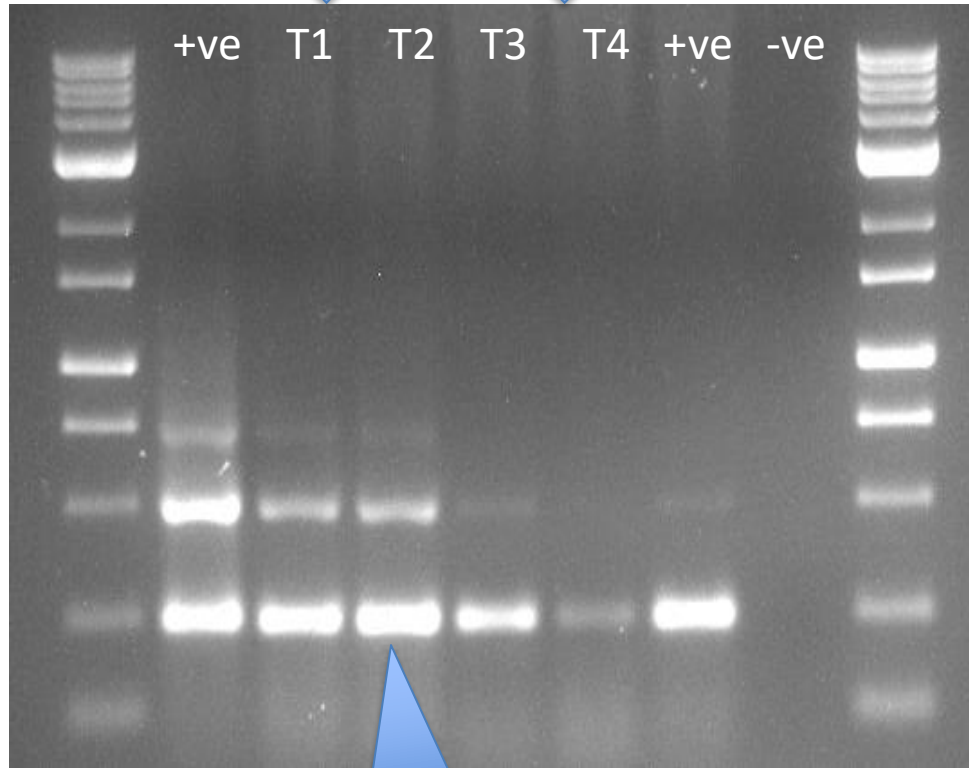


SOME DRIED SAMPLES HAD
BEEN STORED FOR UP TO 18
MONTHS AND HOP LATENT WAS
PRESENT



150 um

75 um

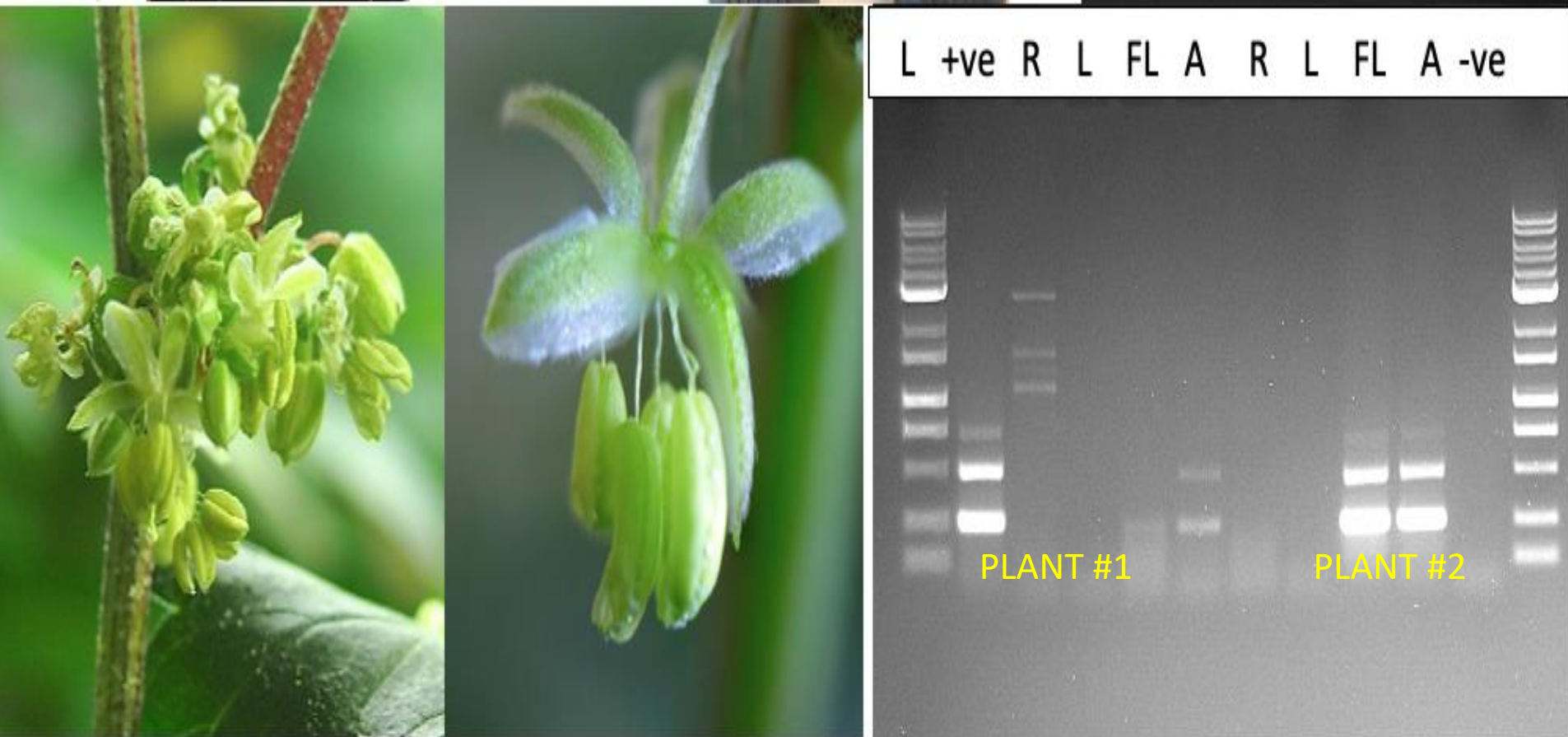


HpLVD is
present in
trichomes !!!

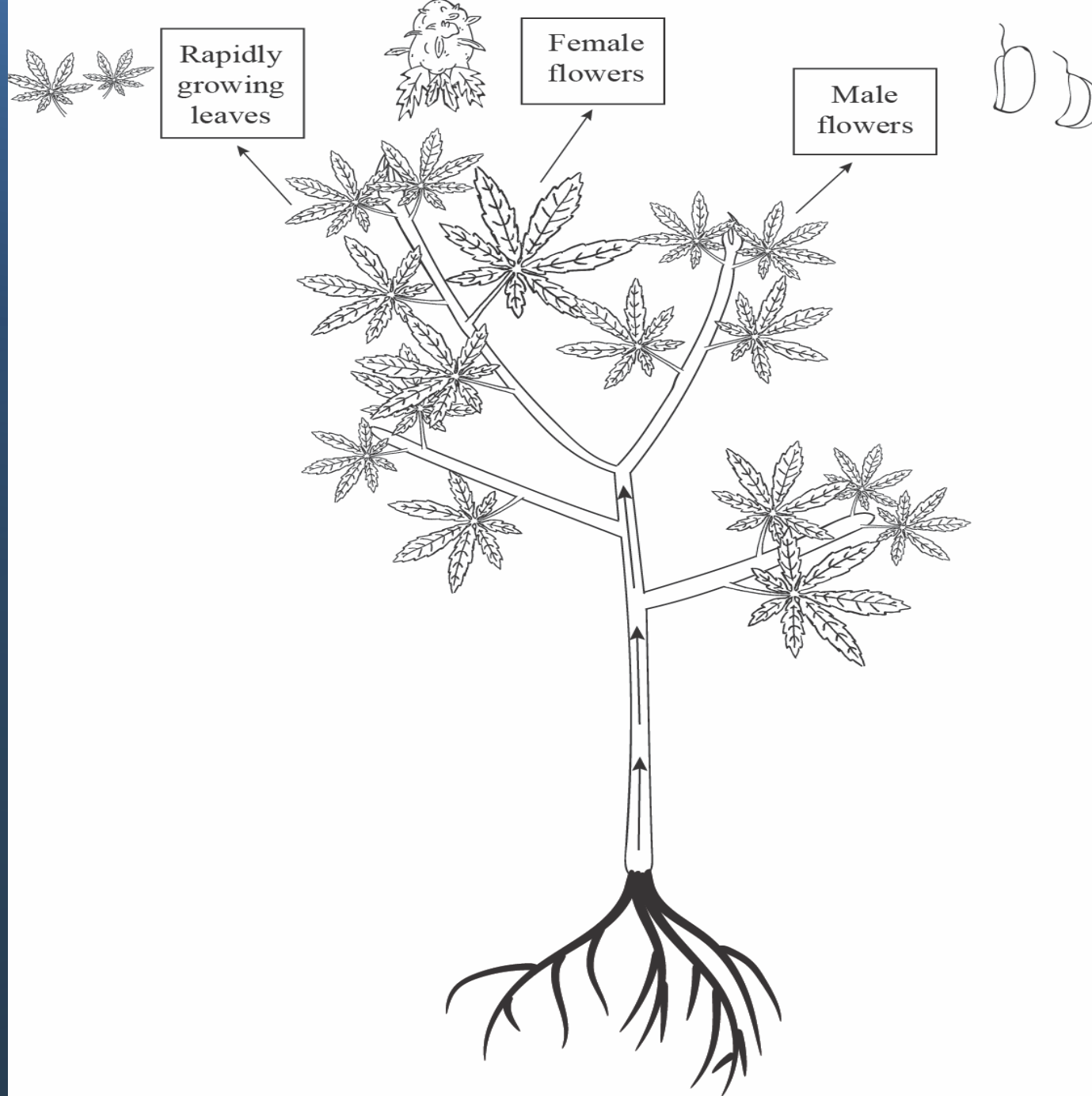


Photo credit : Zoom_Gardens (Nick Cash)

SPREAD ---- Hop Latent viroid can be found in male flowers



The viroid is found in male flowers and in anthers. But UNCONFIRMED in pollen grains



Hop Latent Viroid

IS HOP LATENT VIROID PRESENT IN YOUNG SEEDLINGS DERIVED FROM INFECTED MOTHER PLANTS ?

EXPT. C

HLVd Positive (+) Females



HLVd Negative (-) Male



×



VIROID
PRESENT
ON SEED
COAT AND
INSIDE SEED

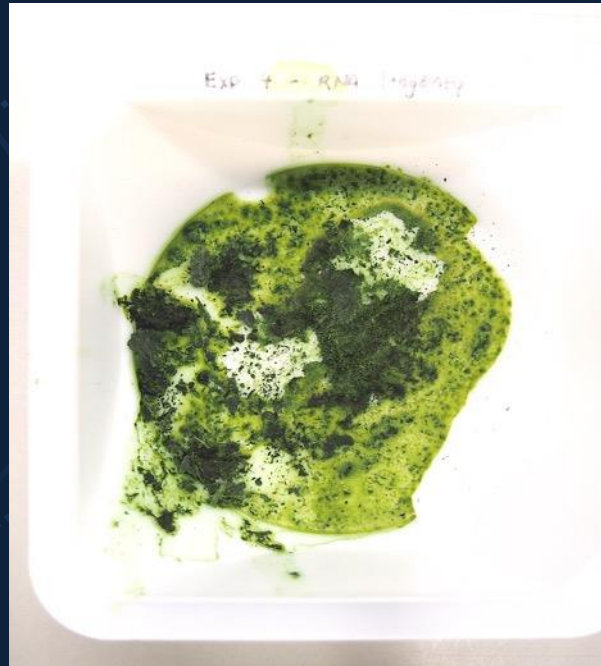
INFECTION RATE = 31/71 SEEDLINGS OR **43.7 %**.

VARIES FROM MOTHER TO MOTHER. RANGE OF 23 % TO 53 %

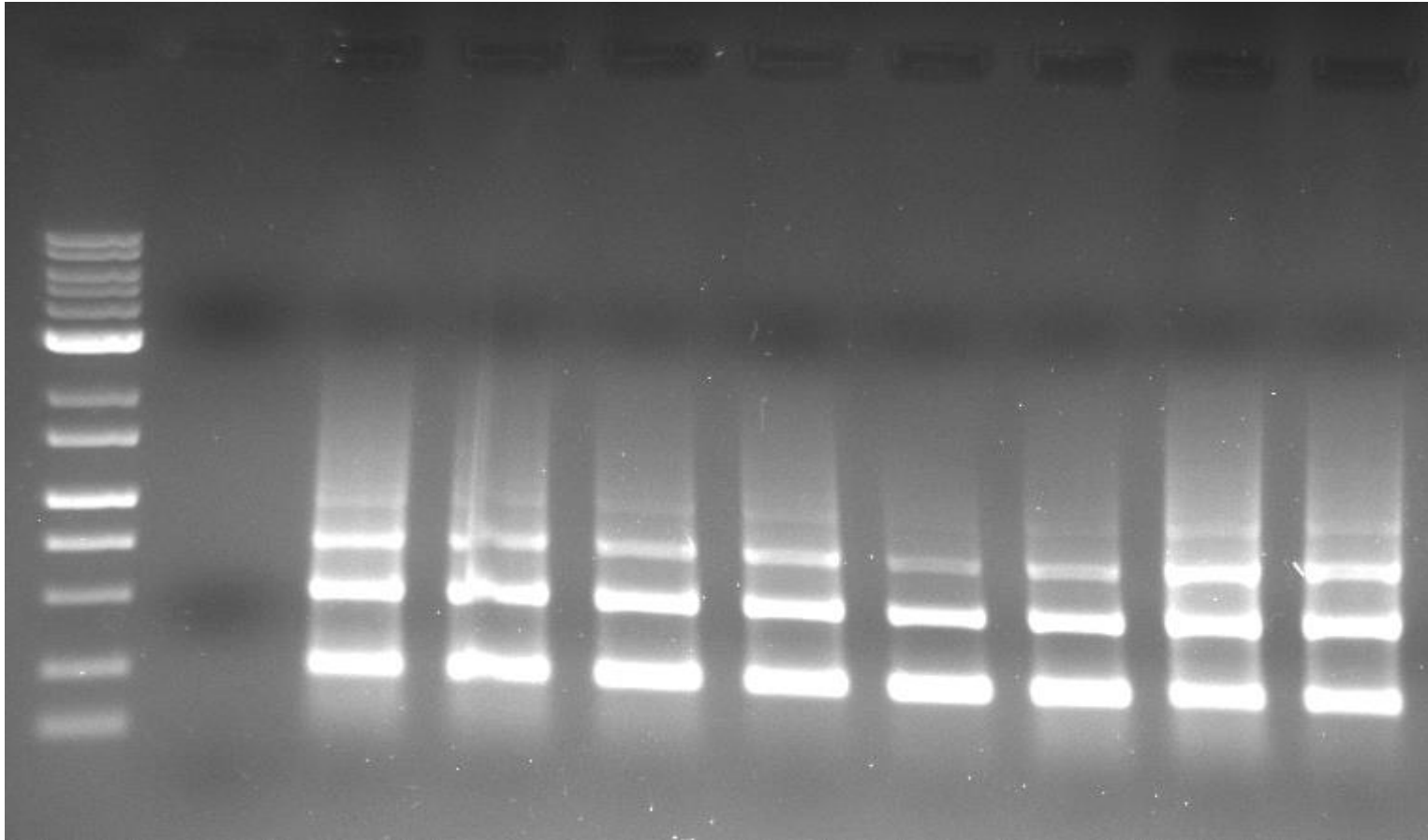
SURVIVAL

. How long is Hop Latent viroid detectable (stable) outside a plant ?

- With dried sap on gloves, allow to sit on a table top for various time periods –
- HpLVd was shown to be stable in dried sap for up to 10 days

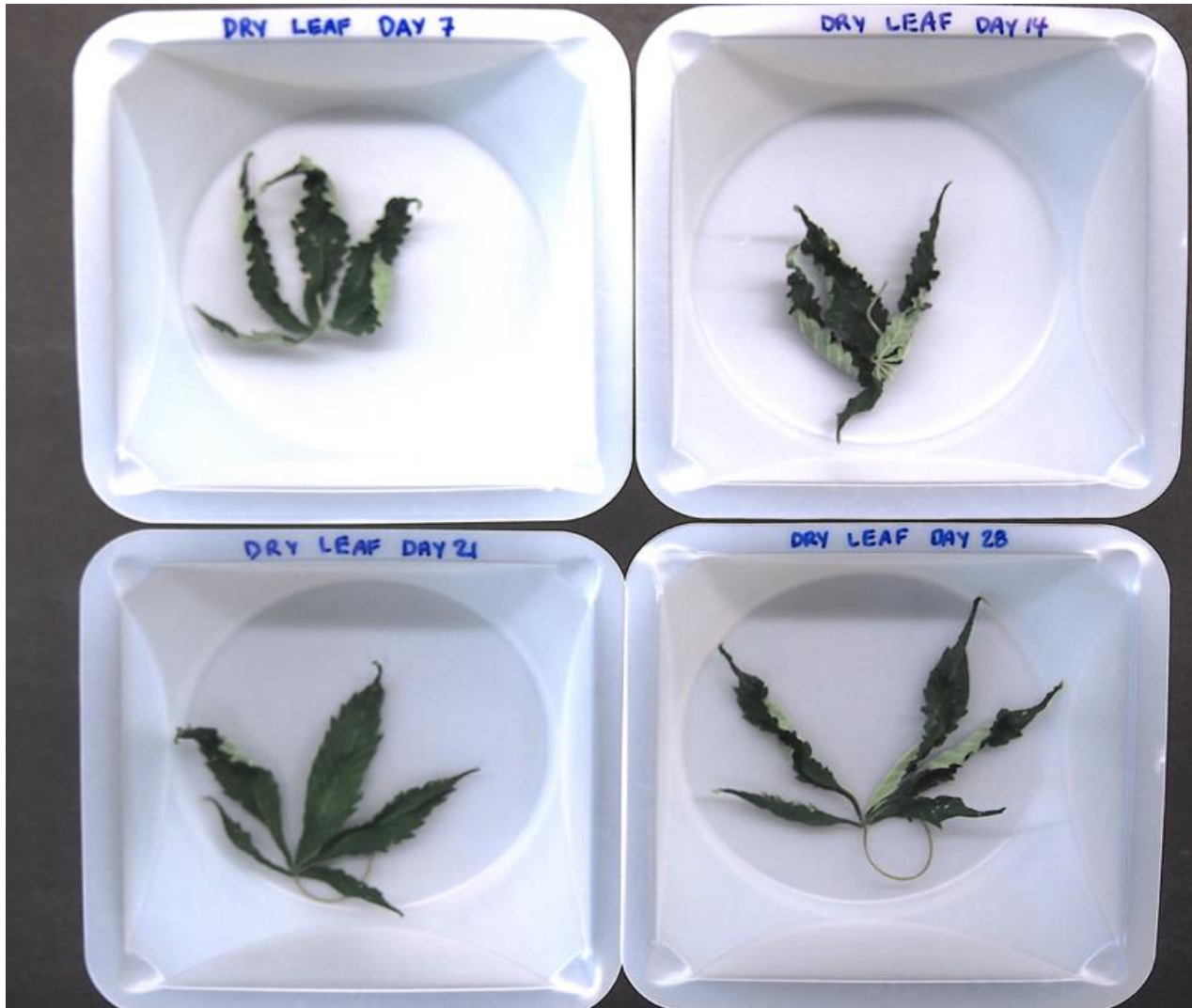


SURVIVAL IN DRIED SAP FOR MORE THAN 7 DAYS



- 1HR 5HR 1D 2D 3D 4D 5D 7D

Survival in dried leaves and sap (test after 1, 2, 3 and 4 weeks)



Results to date show HpLVd can survive in dried leaves for at least 4 weeks

Stability in root tissues.

Infected roots in Petri dishes are exposed to treatments



**Check for viroid stability
after treatments**

1. Temperatures from 30⁰ C to 70⁰ C for 15-30 minutes

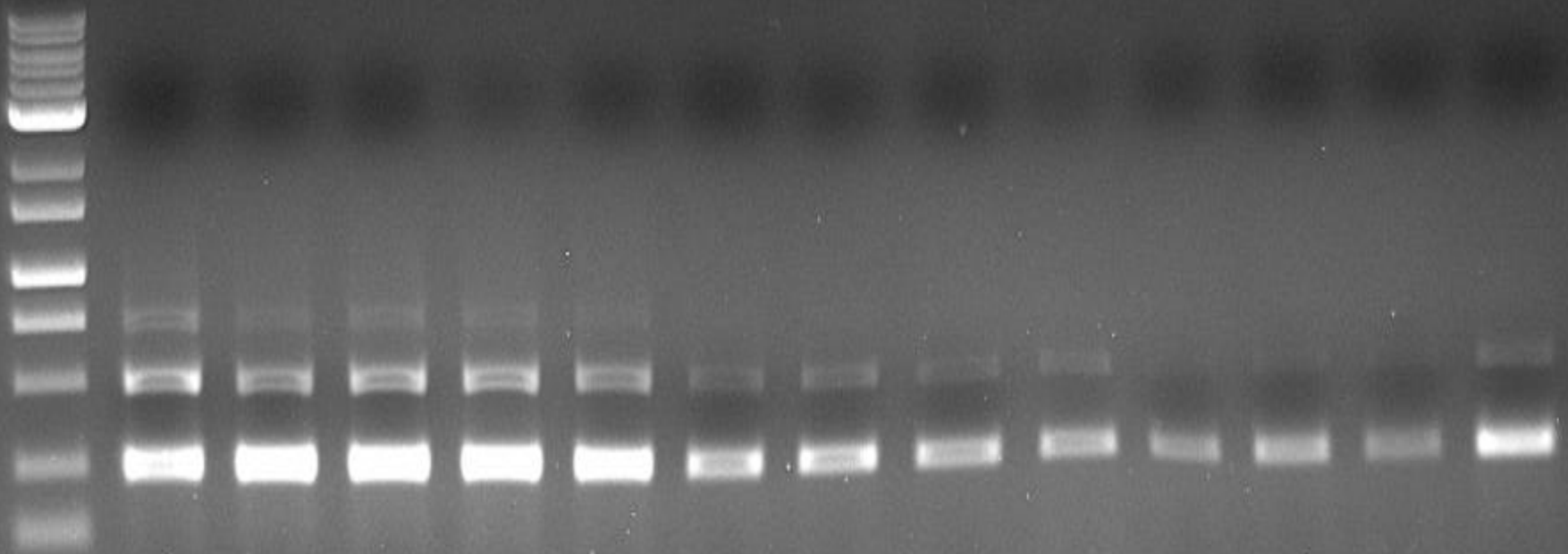
2. At room temperature for 3-12 days

3. Exposure to UV-C for up to 5 min

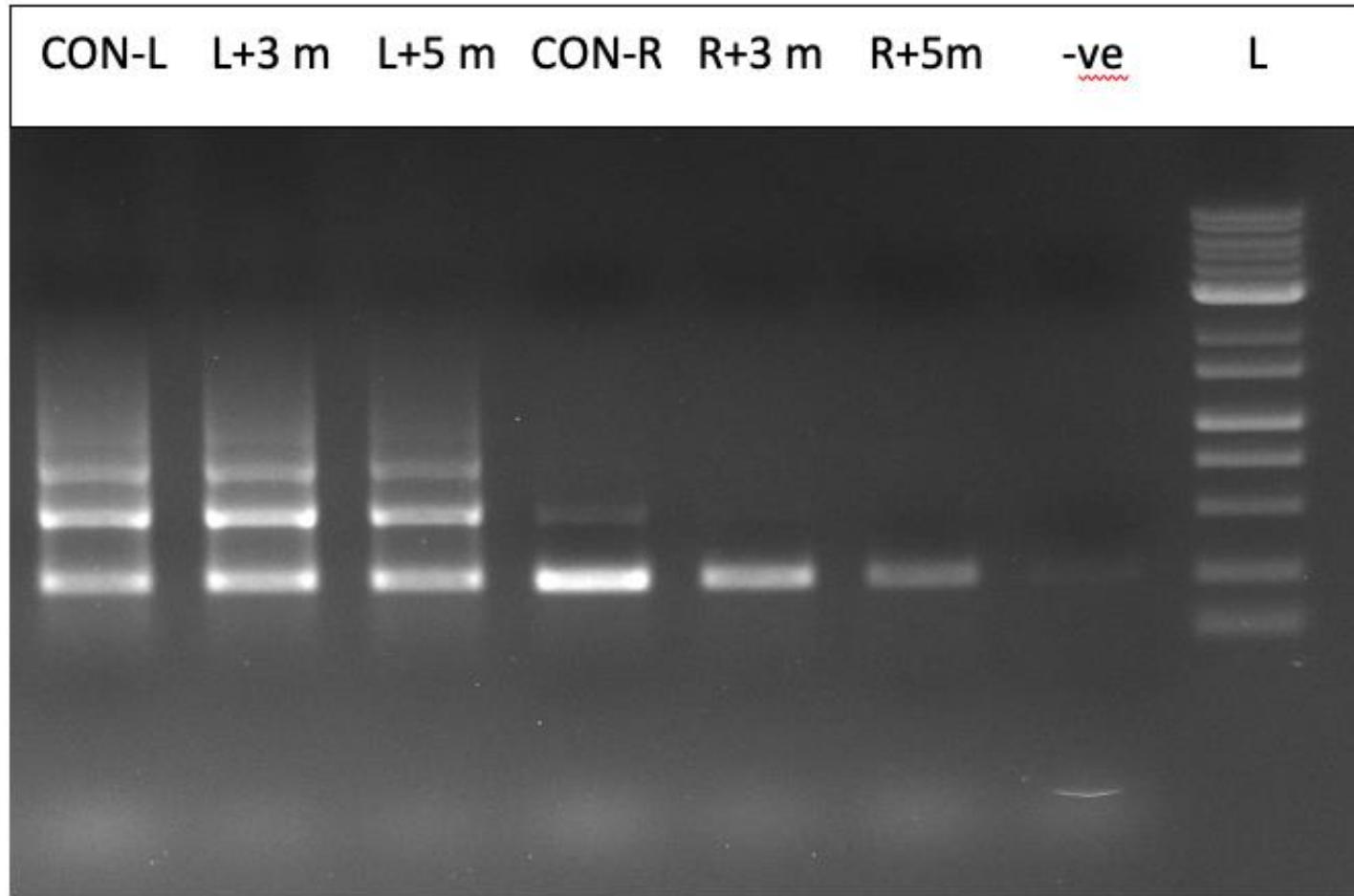
4. Addition of bleach, Virkon, hydrogen peroxide, hypochlorous acid for 2 minutes

STABILITY - Effect of temperature on HpLVd stability in root tissues

| | | | | | | | | | | | | | |
|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|-----|
| L | +VE | CON | 30 C | 30 C | 40 C | 40 C | 50 C | 50 C | 60 C | 60 C | 70 C | 70 C | CON |
| --- | --- | --- | 15 m | 30 m | 15m | 30m | 15m | 30m | 15m | 30m | 15m | 30m | --- |



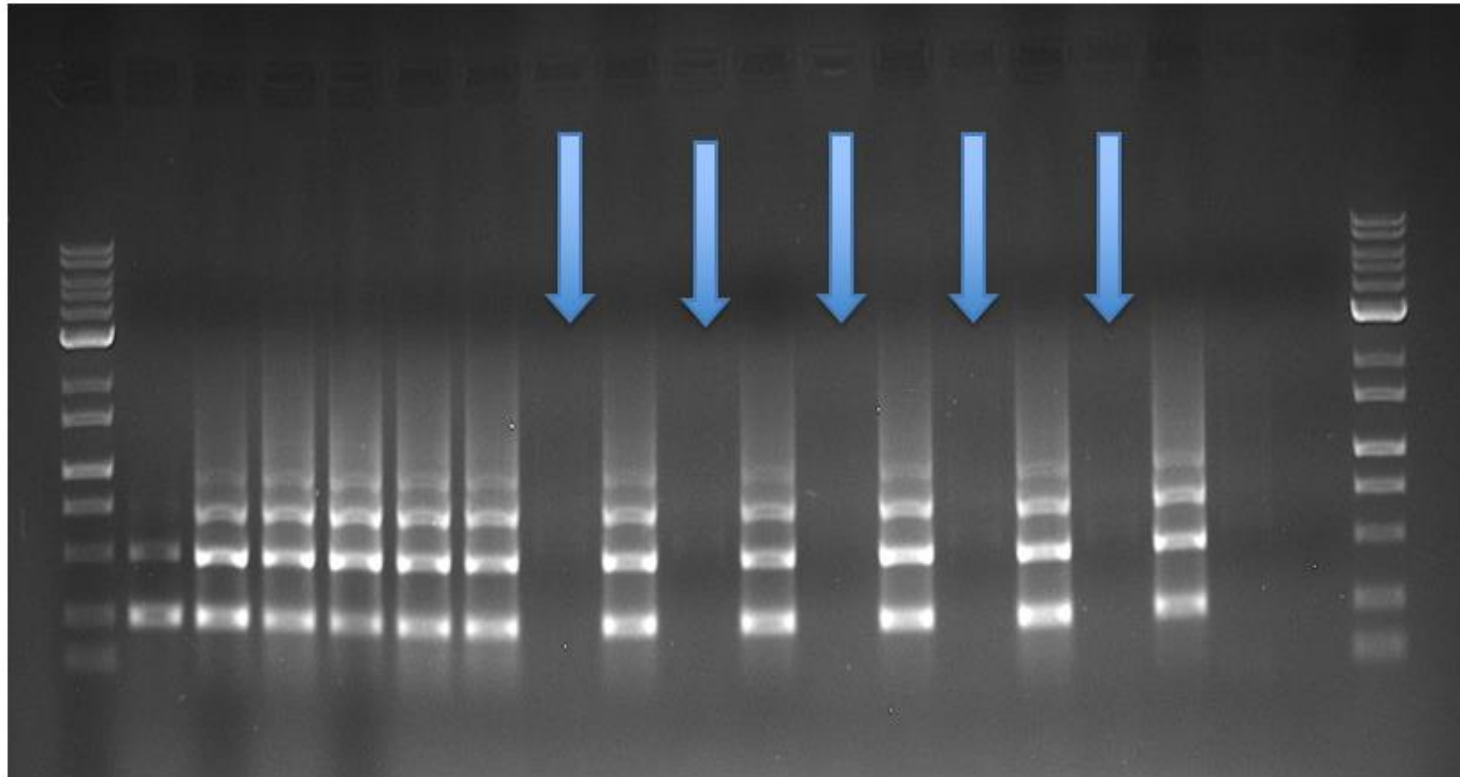
STABILITY - Effect of exposure of leaves and roots for 0, 3 and 5 min to UV-C



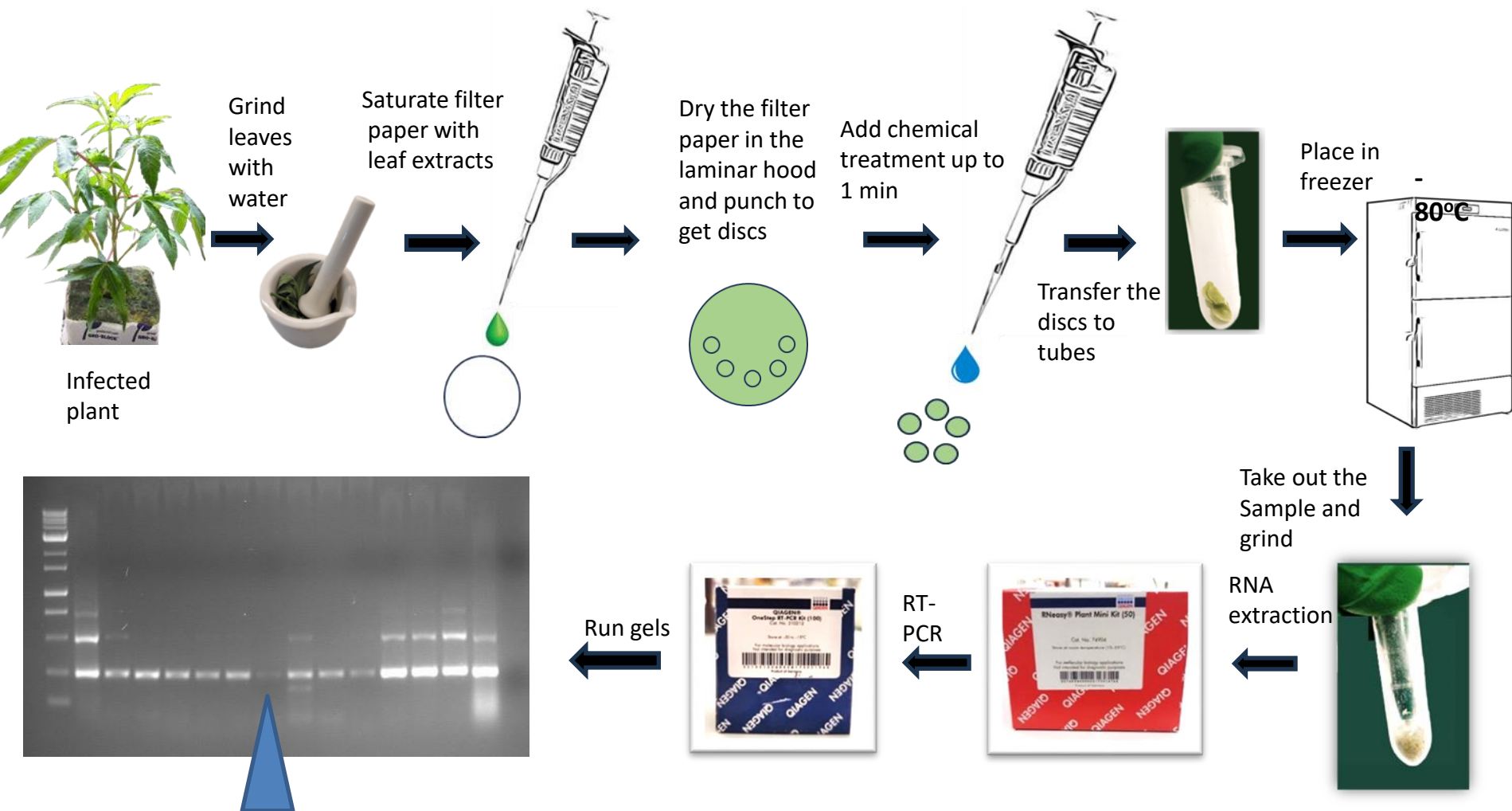
UV-C treatment has no effect on HpLVD in leaves, a slight reduction after 5 min treatment of roots

STABILITY

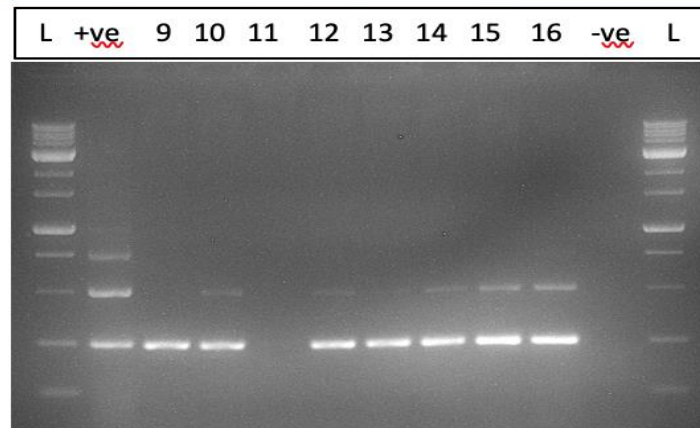
The addition of nucleases (RNase III) destroys the RNA of Hop Latent viroid

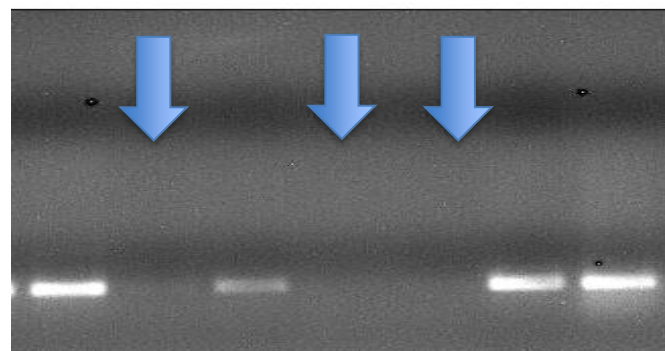
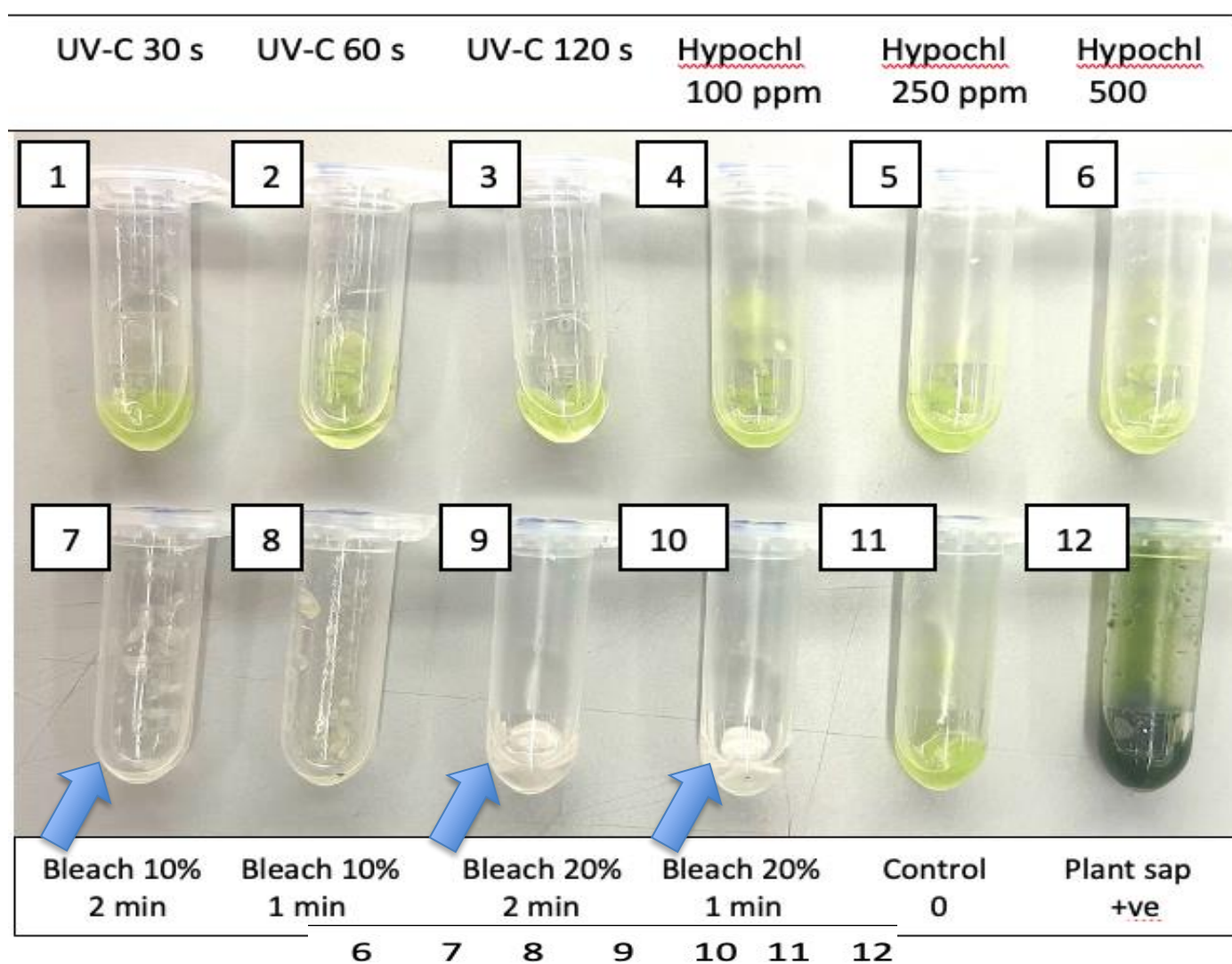


These enzymes are found in plants, bacteria, fungi, yeasts and humans



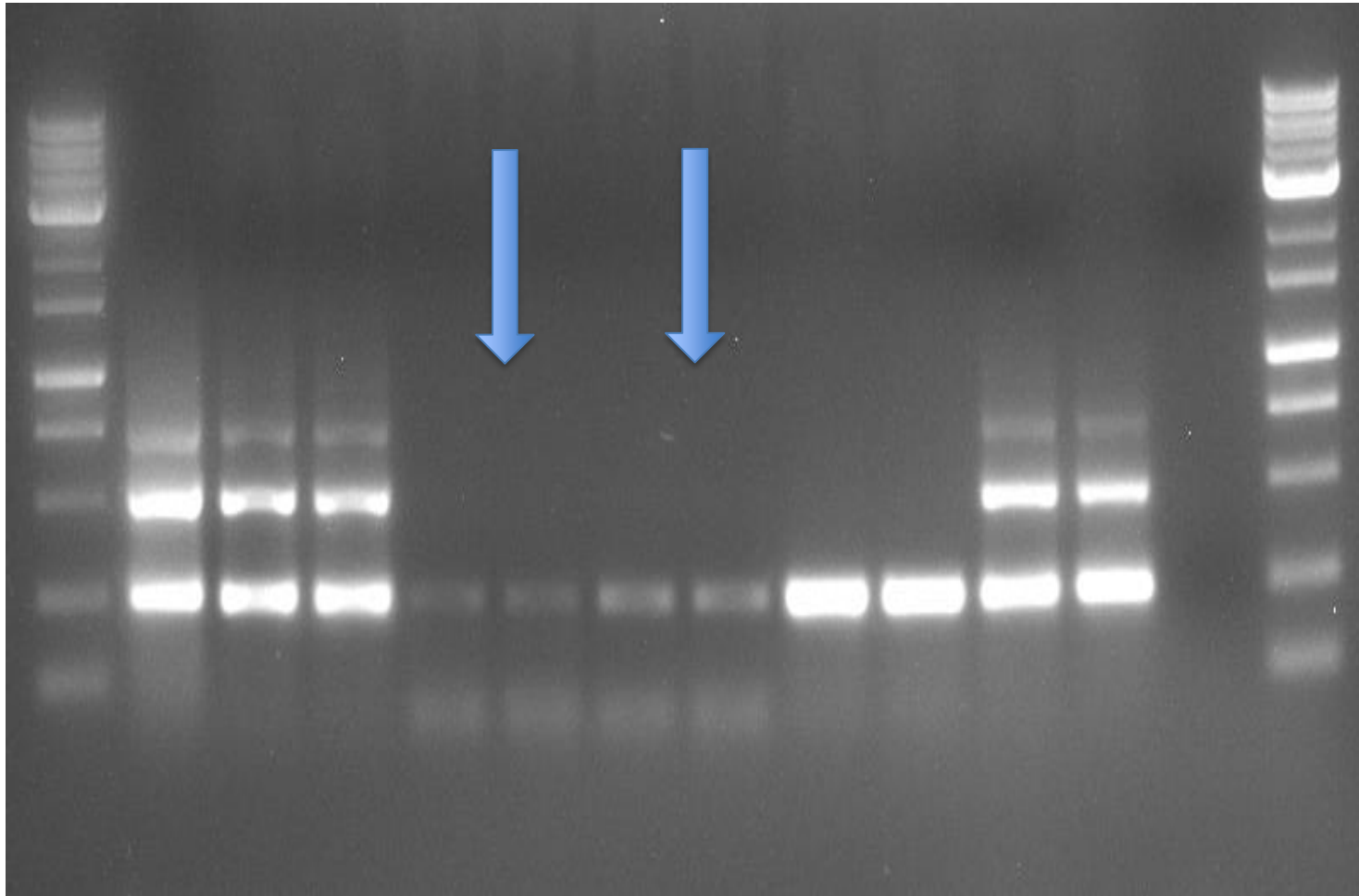
SANITATION





Effect of 10 % bleach and 1,000 ppm hypochlorous acid on Hop latent viroid RNA degradation

UNTREATED BLEACH HYPOCHL. VIRKON UNTREATED



CONCLUSIONS

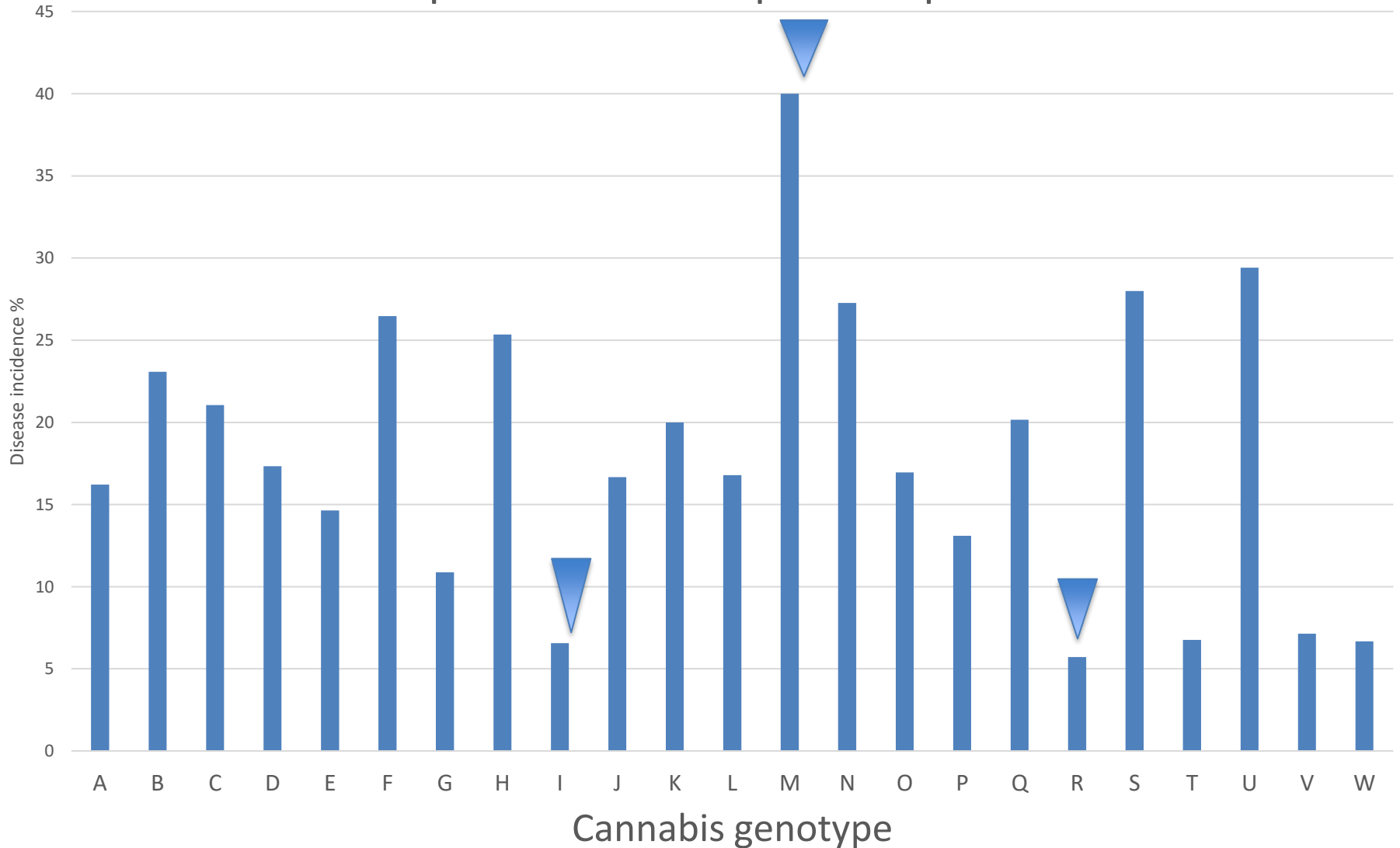
- Hop latent viroid (HpLVd) spreads readily through clones from infected mothers
- Symptoms may not be apparent until plants are in flowering phase
- The viroid spreads into the roots, and then throughout the plant (systemic)
- HpLVd was detected in female flowers, in trichomes, and in male flowers and seeds
- HpLVd causes a reduction of up to 40% in THC levels, depending on the genotype

CONCLUSIONS (Cont.)

- The viroid is very stable in sap, dried leaves
- The viroid is stable at high temperatures
- The RNA of the viroid was destroyed by nuclease enzymes, and by exposure to 10% bleach and 1,000 ppm hypochlorous acid for 1 min.
- Clean planting stock, disinfecting tools, and careful monitoring of infected flowering plants is needed.
- There are significant differences between cannabis genotypes in response to Hop latent viroid

There are obvious differences in Incidence of HPLVd in **cannabis genotypes**

Proportion of HLVD positive plants



Research support acknowledgements

Several Licensed Producers and organizations have made **significant** contributions to this research.

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